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# Role of clay minerals on soil organic matter stabilization and humification

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# Role of clay minerals on soil organic matter stabilization and humification

by

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**For the Major Program**

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# CHAPTER 1
# GENERAL INTRODUCTION

## INTRODUCTION

Soils are composed of solid (50%), liquid (20-30%) and gaseous (20-30% v/v) phases (Brady, 1974). The solid phase can be further divided into the mineral (45% w/w) and organic (5%) fractions (Brady, 1974). The organic fraction is comprised of humic materials, both fresh and partially decomposed plant and animal tissues, and biomass. The mineral fraction is composed of primary and secondary minerals including clay minerals and oxides.

## SOIL ORGANIC MATTER

Soil organic matter (SOM) and soil clay minerals are very important components of a soil because of their chemical and physical properties. SOM is the organic compounds in soil, exclusive of undecayed plant and animal tissues and the soil biomass (Stevenson, 1982). Typically, mineral soils range from less than 1% (sandy soils) up to 10% (poorly drained soils) SOM (Stevenson, 1982). From an agricultural point of view, SOM affects the physical, chemical and biological properties of soils. SOM significantly enhances the stabilization of soil structure, and thereby increases aeration, water-holding capacity and permeability of soils. SOM also indirectly increases nutrient availability to plants via mineralization of organic

forms of N, P, S and other nutrients. From an environmental point of view, SOM reduces transport of organic and inorganic contaminants to groundwater and surface water by binding those contaminants to its network. Moreover, the sequestration of C in SOM has been suggested as one means of reducing the rate of increase of greenhouse gases in the atmosphere.

## SOM Composition

### Humic substances

SOM can be fractionated into humic and nonhumic substances. Humic substances are a series of relatively high-molecular-weight substances formed from secondary synthesis reactions. Humic substances, which constitute from 65 to 80% of the SOM (Schnitzer, 1986; Senesi and Loffredo, 1999), can be fractionated into fulvic acid, humic acid and humin using strong alkali and acid solutions. Fulvic acid is soluble under both alkaline and acidic conditions. Fulvic acids are light in color and have a lower molecular weight, lower C content, higher O content, and higher total acidity than humic acids (MacCarthy et al., 1990; Malcolm, 1990; Stevenson, 1982). Humic acid is the dark-colored fraction that is soluble in alkaline solutions but insoluble under acidic conditions. The molecular weight of humic acids is greater than that of fulvic acid, the C and O contents are about 60 and 30%, respectively, and the estimated total acidity is 8.1 meq $g^{-1}$ (Stevenson, 1982). The humic/fulvic acid ratios vary substantially from soil to soil ranging from 0.3 (tundra soils) to 2.5 (prairie soils) (Stevenson, 1982). Humin is the SOM fraction that is insoluble in both alkali and acid, and "is believed to exist as high-molecular-weight polymers associated with sesquioxides and silicates" (Stevenson, 1982).

**Non-humic Substances**

Non-humic substances are partially decomposed plant and animal tissues. Non-humic substances include all known classes of biochemical compounds such as proteins, carbohydrates, lipids, organic acids, among others (Stevenson, 1982). Non-humic substances constitute from 20 to 35% of the SOM (Schnitzer, 1986; Senesi and Loffredo, 1999). The average composition of non-humic substances is: carbohydrates, 30%; N-containing compounds (proteins, peptides, amino acids, amino sugars, purines, pyrimidines, and unidentified compounds), 30%; lipids (alkanes, fatty acids, waxes, and resins), 40% (Senesi and Loffredo, 1999).

**SOM synthesis**

There are four major theories for the formation of humic substances. The oldest theory assumes that lignin is the precursor material for the formation of humic substances. This theory proposed that lignin is partially used by organisms, and the modified lignin residue becomes part of the SOM (Stevenson, 1982; Stevenson and Cole, 1999). The second theory assumes that lignin is used by organisms and the "by-products" which are phenolic aldehydes and acids are condensed and polymerized to form humic substances (Stevenson, 1982; Stevenson and Cole, 1999). The third theory also assumes that polyphenols are the building blocks of humic substances; however, the starting materials are hypothesized to be microbial transformation products from non-lignin C sources. These polyphenols are oxidized to quinones and ultimately polymerized to form humic substances (Stevenson, 1982; Stevenson and Cole, 1999). The fourth theory proposes the polymerization of reducing sugars and amino acids to form brown nitrogenous polymers. The starting

materials are assumed to be by-p roducts of the microbial metabolism (Stevenson, 1982; Stevenson and Cole, 1999).

## CLAY MINERALS

Soil clay minerals can been grouped into 1:1 and 2:1 clay minerals. The 1:1 clay minerals, primary kaolinite, are found in most soils, but predominate in highly weathered soils of humid temperate and tropical regions (Schulze, 2002). The 2:1 clay minerals are grouped into non-swelling and swelling clays. The former have a high negative surface charge, however, swelling is inhibited by interlayer $K^+$ that is held tightly in the hexagonal siloxane cavities. The 2:1 swelling or expandable clays include the smectite and vermiculite groups. Smectites have large surface areas, high cation exchange capacities (CEC) and are classified as montmorillonite (dioctahedral, Mg-Al octahedral substitution), beidellite (dioctahedral, Al-Si tetrahedral substitution), nontronite (Fe-rich, dioctahedral, Al-Si tetrahedral substitution), hectorite (Mg-rich, trioctahedral, Li-Mg octahedral substitution), saponite (Mg-rich, trioctahedral, Al-Si tetrahedral substitution), and sauconite (Zn-rich, dioctahedral, Al-Si tetrahedral substitution) (Reid-Soukup and Ulery, 2002).

### Surface acidity

Smectites behave as Lewis acids when incompletely coordinated Al and Fe ions are exposed at the broken edges or as hydrated interlayer cations (Heller-Kallai, 2002; Solomon and Hawthorne, 1983). When hydrated, exposed non-bridging OH groups tetrahedrally or octahedrally coordinated with Si, Al, and Fe are weak Brönsted acids (Heller-Kallai, 2002; Solomon and Hawthorne, 1983). In hydrated

systems the acidic character of smectites increases with the polarizing power of the exchangeable cations (decreasing size and increasing charge). Furthermore, as the number of water molecules coordinated to the exchangeable cations decreases, the remaining water molecules become increasingly polarized and are thus better able to donate protons (Heller-Kallai, 2002; Solomon and Hawthorne, 1983).

## IRON OXIDES

Iron oxides, hydroxides, and oxyhydroxydes are widespread components of soils. Goethite ($\alpha$-FeOOH), the most common Fe oxyhydroxide in soils, consist of double chains of Fe octahedrally coordinated to O and OH. The doubled chains are bound to neighboring double chains by Fe-O-Fe and H bonds and separated by double chains of vacant sites that form tunnels (Bigham et al., 2002; Schwertmann and Taylor, 1989). Soil iron oxides have a pH dependent or variable surface charge because of the weakly acid surface FeOH groups (Schwertmann and Cornell, 1996). Three types of OH groups are found on the *(001)* surface of goethite: Type A OH's coordinate with only one Fe atom and readily participate in ligand exchange reactions. Type B OH's and C OH's coordinate with two Fe atoms and generally form hydrogen bonds with the ligands (Zhang and Yu, 1997). Goethites and Al-substituted goethites can easily be synthesized in the laboratory from $Fe(NO_3)_3$ solutions (Schwertmann and Cornell, 2000).

# CATALYTIC POLYMERIZATION OF ORGANIC COMPOUNDS

## Biotic catalytic polymerization

The polyphenol theory for the formation of humic substances from non-lignin C source has been widely studied by several researchers. Both biotic and abiotic catalyzed polymerization have been studied. Microorganisms are capable of polymerizing phenolic compounds into humic-like substances (Haider and Martin, 1970; Liu et al., 1981; Martin et al., 1972; Pal et al., 1994; Saiz-Jimenez et al., 1975; Sulfita and Bollag, 1981). For example, phenoloxidase, an nzymes present in soils, can biotically catalyze the formation of humic-like substances from naphthols (Sulfita and Bollag, 1981) and lignin phenol derivatives (Liu et al., 1981; Martin et al., 1972). Furthermore, laccase, a *p*-diphenol oxidase from fungi, was shown to be more effective for oxidizing phenols than abiotic-catalyzed reactions with birnessite, a manganese oxide (Pal et al., 1994).

The nature of N-containing compounds used as N source in the biotically catalyzed polymerization of phenols influenced the N content in the humic-like substances. Using $NaNO_3$ as a N source only, only 1% humic-like substances were formed compared with 4.5% N when asparagine was used as a N source (Saiz-Jimenez et al., 1975). It has been shown that the reactions of phenols with amino acids, peptides, and proteins in the presence of laccase form humic-like polymers and that deamination and decarboxylation of amino acids occurred (Haider et al., 1965). Moreover, the reaction products of amino acids with phenols were stable against acid hydrolysis, whereas the reaction products of peptides with phenols could be hydrolyzed except for phenol-bounded amino groups (Haider and Martin,

1970; Haider et al., 1965).

## Abiotic catalytic polymerization

### Role of Clay Minerals

Smectites (montmorillonite and nontronite), illite and kaolinite have been reported to serve as catalysts for the formation of humic-like substances from mixtures of phenols (Wang and Huang, 1989; Wang, 1987; Wang et al., 1978; Wang et al., 1980; Wang et al., 1983). The rate of phenol polymerization or formation of humic-like substances from phenolic compounds is affected by: (1) the clay mineral type: smectite > illite > kaolinite > quartz (Wang et al., 1978); (2) the type of smectite: nontronite > montmorillonite (Wang and Huang, 1989); (3) the nature of the phenols: vicinal trihydroxy > vicinal dihydroxy > monohydroxy (Wang et al., 1983); (4) and pH: neutral > alkaline > acidic (Wang et al., 1980). Under acidic conditions, illite abiotically catalyzed the formation of fulvic acid-like compounds whereas under alkaline conditions the formation of humic acid-like compounds was favored. However, when $CaCO_3$ was added to the illite suspensions (pH 8.5), the formation of fulvic acid was favored (Wang et al., 1980).

The condensation of phenols with amino acids by clay minerals has been studied. Nontronite has been shown to enhance the rate of condensation of glycine and phenols and to catalyze the deamination and decarboxylation of glycine (Wang, 1991; Wang and Huang, 1991). Electron spin resonance spectroscopy (ESR) has shown that phenol polymerization occurs via radical formation on exposed $Fe^{3+}$ sites on the edges of nontronite, a $Fe^{+3}$-rich smectite, and chemisorbed $O_2$ on clay minerals (Wang and Huang, 1989; Wang and Huang, 1991). Furthermore,

polymerization of aromatic compounds via radical formation on $Cu^{2+}$-saturated montmorillonite and hectorite has been also reported (Boyd and Mortland, 1986; Mortland and Halloran, 1976; Porter et al., 1997).

Polymerization of amino acids by clay minerals, in particular smectites, has been studied extensively. Yields of peptides from clay mineral + amino acid systems formed during drying-wetting cycles at relatively high temperatures are affected by several factors. The type of clay mineral affects the rate of polymerization, with; smectite > chlorite > vermiculite > kaolinite for glycine polymerization, and trioctahedral smectite > chlorite > dioctahedral smectite > kaolinite for diglycine polymerization (Bujdák and Rode, 1999). The layer charge location effects the rate of polymerization, with octahedral > tetrahedral (Bujdák and Rode, 1996). The saturating cation on montmorillonite effects the rate of alanine-adenylate, $Na^{+} > Al^{3+}$, (Paecht-Horowitz and Lahav, 1977), and $Ca^{2+} > Cu^{+2}$ for glycine polymerization (Bujdák et al., 1994; Bujdák et al., 1995). Other factors influencing the rate of polymerization include the type of amino acid (glycine and glycine peptides > alanine peptides) (Bujdák et al., 1995), temperature and water content (wetting-drying cycles and temperature fluctuations > temperature fluctuations alone) (Lahav et al., 1978), and initial pH (5 > 4 > 3 for Ca-montmorillonite + diglycine systems ) (Bujdák et al., 1996).

The non-enzymatic condensation of reducing sugars and amino acids to form melanoidins (insoluble brown nitrogenous containing compounds) is known as the Maillard reaction. This reaction is hypothesized to occur in soils to form humic-like substances (Stevenson, 1982). Maillard reaction studies have been conducted

under "wetting-drying" cycles at relative high temperatures (70 to 100 °C) mimicking pre-biotic conditions on the earth. The type of clay mineral influences the rate of formation of melanoidins from D-glucose with amino acids (tyrosine, glycine, and tryptophan); smectites > kaolinite (Arfaioli et al., 1997; Arfaioli et al., 1999; Bosetto et al., 2002; Bosetto et al., 1994). In addition, it has been shown that clay minerals (smectites and kaolinites) form "more complex" or aromatic substances than quartz in amino acid + glucose systems as elucidated from the C:N and the 465 nm/665 nm absorbance ratios of the condensation products (Arfaioli et al., 1997; Arfaioli et al., 1999; Bosetto et al., 2002; Bosetto et al., 1994). Higher melanoidin yields were formed in quartz + amino acid (glycine or tyrosine) + glucose systems than in clay mineral systems when the same amount of saturating cations ($Ca^{2+}$, $Cu^{2+}$, or $Al^{3+}$) in clays were added to quartz, suggesting that the amount of cations in solution influences the formation of melanoidins (Arfaioli et al., 1997; Arfaioli et al., 1999; Bosetto et al., 2002). In addition, $Cu^{2+}$ in solution in the quartz + tryptophan + glucose system formed more condensation products than were formed in the $Cu^{2+}$-saturated clay minerals systems. However, when $Ca^{2+}$ and $Al^{3+}$ where used as saturating cations, the clay systems formed melanoidins, whereas melanoidin formation in the Ca- and Al-quartz systems was negligible (Arfaioli et al., 1997; Bosetto et al., 1994). Furthermore, the formation of melanoidins during the incubation of glucose + amino acids increased for acidic < neutral < basic amino acid (Hedges, 1978) suggesting that the type of amino acid present in the system plays an important role on the formation of melanoidins.

Maillard reactions also occur in protein + reducing sugar systems. In the

reaction of casein (a milk protein) with glucose, arginine content in the fulvic acid-like fraction decreased with time of reaction from 28 to 4% (% mol) of the total amino acid content after 2- and 7-day incubations, respectively (Yamamoto and Ishiwatari, 1989). Interestingly, arginine content in the initial milk casein was about 3% of the total amino acid content, suggesting that arginine is very reactive towards glucose (Yamamoto and Ishiwatari, 1989). In studies of $Ca^{2+}$- and $Al^{3+}$-clay mineral + amino acid (tryptophan and tyrosine, aromatic amino acids) systems, under similar conditions, higher yields of melanoidins were obtained for smectites than for kaolinites (Bosetto et al., 1995; Bosetto et al., 1997). The melanoidins formed in quartz + tryptophan systems were negligible (Bosetto et al., 1995); however, when $Cu^{2+}$ was added to quartz + tyrosine systems, the melanoidin yields were greater than for clay mineral systems (Bosetto et al., 1997).

Transformations of glucose are influenced by pH. Glucose oxidizes to glucuronic acid under alkaline conditions, whereas under acidic conditions glucose is dehydrated to form furfural compounds (Theander, 1988). Furfural compounds are intermediate compounds for the most advanced stages of the Maillard reaction (Ferrer et al., 2000). The most important furfural compounds in the Maillard reaction are hydroxymethylfurfural (HMF), furfural, furylmethylacetone, and methylfurfural (Ferrer et al., 2000). Pillared montmorillonites have been shown to facilitate four acid-catalyzed reactions involving glucose; isomerization of glucose to fructose, dehydration of glucose to HMF, cleavage of HMF to formic acid and levulinic acid and formation of humified material (Lourvanij and Rorrer, 1994; Lourvanij and Rorrer, 1997).

**Role of Oxides and Hydroxides**

Only a few studies have been conducted on the role of metal oxides as catalysts in the formation of humic-like substances. The type of metal oxides has been shown to affect the yield of polymerization products of phenolic compounds; with $MnO_2$ > $Fe(OH)_3$ > $Fe_2O_3$ > $Al_2O_3$ (Lehmanh et al., 1987). The importance of metal oxides in soils on the polymerization of phenols also has been demonstrated. The removal of free oxides (Fe, Al and Si) from soil clay fractions decreased the catalytic power of the clay fractions to polymerize phenolic compounds (Wang et al., 1978; Wang et al., 1983). Catechol oxidation is catalyzed by alumina, an Al oxide (Wang, 1983), and birnessite, a Mn oxide (Naidja et al., 1998).

There is lack of information of the role of Fe oxides on the catalytic polymerization of amino acids; however, a few studies have been conducted using other metal oxides. Alumina and silica (Al and Si oxides, respectively) are more efficient for amino acid dimerization than clay minerals under "wetting-drying" cycles at 80 °C (Bujdák and Rode, 1997). Alanine oligomerization was abiotically catalyzed by three types of alumina, under "neutral" > "basic" and > "acidic" conditions over a wide range of temperatures (Basiuk and Sainz-Rojas, 2001). Moreover, the type of amino acid influenced peptide yields when alumina was used as a catalyst; glycine > alanine > leucine > valine > proline (Bujdák and Rode, 2002).

## HYPOTHESIS AND OBJECTIVES

Although there is a vast amount of information on the role of clay minerals in

catalyzing the formation of melanoidins under geologically relevant conditions, there is little information on the ability of smectites to abiotically catalyze the condensation of amino acids and reducing sugars under conditions found in soil environments.

Because of the chemical properties of smectites, they can catalyze numerous organic reactions; including Diels-Alder cycloadditions (Heller-Kallai, 2002; Laszlo, 1987), Friedel-Crafts reactions (Heller-Kallai, 2002; Laszlo, 1987), aldol reactions (Izumi, 1992), Michael additions reactions (Izumi, 1992) and oxidation reactions (Heller-Kallai, 2002). Thus, it is reasonable to hypothesize that soil smectites are catalysts for some if not most of the organic reactions involved in the formation of humic substances.

From 32 to 50% of the total extracted N from soils was found to be amino acid N (Senwo and Tabatabai, 1998). Furthermore, the amino acid N and amino sugar N content accounted for 76-97% and 4 to 6%, respectively, of the total N in the clay fractions of a Webster soil (Laird et al., 2001). Arginine, a basic amino acid, accounted for 50 to 66% of the total extractable N in these soil clay fractions (Laird et al., 2001). Soil carbohydrate content varies from soil to soil. In general, cellulose and derivatives account for up to 15% of the total SOM, whereas hexoses, pentoses, uronic acids and amino sugars account for 4-12, <5, 1-5, and 2-6% of the total SOM, respectively (Stevenson, 1982). Glucose, the most abundant monosaccharide in soils, accounts for about 25% of the monosaccharide content (Larre-Larrouy and Feller, 1997; McGrath, 1973) and almost 9% of the total soil C content (Larre-Larrouy and Feller, 1997).

Maillard reaction products or melanoidins have been suggested as one of the

pathways for the formation and polymerization of humic substances. The starting materials, reducing sugars and amino acids, and the catalysts, clays and saturating cations, for these reactions exist in soils. Furthermore, reducing sugars and amino acids are found in the fine clay fractions, where expandable 2:1 clay minerals dominate. If amino acids and reducing sugars are sorbed on clay surfaces; then, it is possible that the Maillard reaction is catalyzed by 2:1 clay minerals in soils. Moreover, Fe oxides are commonly found in soils both as discrete particles and as coatings on clay surfaces; hence, it is possible that Fe oxides also catalyze Maillard reactions in soils. Therefore, clay minerals and Fe oxides are hypothesized to catalyze the abiotic condensation of amino acids and sugars, under conditions similar to those found in soil environments.

The theory that Maillard reaction products are precursors for the formation of humic substances in soils is not widely accepted. However, evidence that Maillard reactions are abiotically catalyzed by clay minerals and oxides would increase understanding of the role of soil clay minerals in the synthesis and stabilization of humic substances in soils. While no single chemical reaction can explain the synthesis of humic substances, a multistage process can be proposed where both biotic and abiotic processes and reactions lead to the formation of polyphenols and melanoidins, and their co-polymerization leads to the formation of humic substances.

The general objectives of this dissertation are: (1) to quantify the dynamics of newly formed humic substance in soil clay fractions, (2) to evaluate the ability of 2:1 expandable clay minerals and Al-substituted goethites to abiotically catalyze the condensation of glucose, (3) to evaluate the ability of 2:1 expandable clay minerals

and Al-substituted goethites to abiotically catalyze condensation of amino acids + glucose, and (4) to elucidate the role of different saturating cations on the abiotic clay-catalyzed condensation of amino acids and glucose.

## DISSERTATION ORGANIZATION

This dissertation presents four studies. Each study was prepared as a complete article in a format acceptable for publication in a scientific journal. In the second chapter, the dynamics of newly formed humic substances in a Monona soil is studied. The $^{14}C$ activity was measured in the fine, medium, and coarse clay fractions of a Monona soil after been incubated for 360 days with $^{14}C$-labeled plant residues under simulated no-tillage management conditions. In the third chapter, the abiotic polymerization/transformation of glucose by four smectites and four synthetic Al-substituted goethites was studied using a 21-day incubation at 37°C. The fourth chapter reports an incubation study of arginine + glucose condensation in the presence of four smectites and four synthetic Al-substituted goethites. The fifth chapter reports an incubation study of diglycine and triglycine + glucose with four synthetic Al-substituted goethites. The final chapter presents general conclusions for the four studies. An appendix includes unpublished and raw data for the four studies.

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# CHAPTER 2

# CARBON SEQUESTRATION IN CLAY MINERAL FRACTIONS FROM $^{14}C$-LABELED PLANT RESIDUES

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## ABSTRACT

An understanding of organic C dynamics in soils is necessary to develop management options to enhance soil organic C sequestration. The objective of this research was to study the distribution of newly formed humic materials into mineralogical distinct clay-size fractions of a silt loam soil. Oats (*Avena sativa L.*), grown under simulated no-tillage conditions, were pulse labeled with $^{14}CO_2$. After senescence, the surface residue was removed and the labeled roots were allowed to decompose in the soil for 360 days. The soil clay fraction (<2 μm) was separated into coarse, medium, and fine clay size-fractions (0.2-2.0, 0.02-0.2 and <0.02 μm, respectively) by centrifugation. X-ray diffraction indicated that quartz, illite, and kaolinite were the dominant mineral phases in the coarse clay fraction while smectite was the dominant mineral phase in the fine clay fraction. The organic C content in the coarse and fine clay fractions (3.70 and 3.93%, respectively) were similar. Scintillation analysis indicated an increase in $^{14}C$ specific activity in all

fractions after 360 days of incubation. For both sampling times, 0- and 360-days, the highest $^{14}C$ specific activity occurred in the fine clay fraction (847.2 and 1529 Bq $g^{-1}$ fraction, respectively), whereas the lowest $^{14}C$ specific activity occurred in the coarse clay fraction (565.8 and 770.9 Bq $g^{-1}$ fraction, respectively). The results suggest that new humic materials are preferentially forming or accumulating on smectite surface.

## INTRODUCTION

The soil C cycle has a substantial influence on the ability of soils to supply air, water, and nutrients to growing plants. Furthermore, concerns about the effect of increasing concentrations of greenhouse gases in the atmosphere on global climate have increased interest in the soil C cycle with a focus on the potential for increasing soil C sequestration. The transformation of plant residues into stabilized clay-humic complexes is a key process influencing both soil quality and whether a soil is a net sink or source of C to the atmosphere. This study was undertaken to further our understanding of the role of soil clay minerals in the formation of humic substances.

Carbon distribution and its biochemical properties have been extensively studied on soil clay size fractions. The coarse clay fractions typically contain more aromatic and recalcitrant or humified organic matter, whereas fine clay fractions contain more labile or less humified organic matter (Anderson et al., 1981; Anderson, 1984; Catroux and Schnitzer, 1987; Christensen, 1986; Christensen, 1987; Christensen and Sorensen, 1985; Laird et al., 2001; Oades, 1995; Turchenek and Oades, 1979). Typically the C:N ratio decreases with particle size (Anderson et

al., 1981; Catroux and Schnitzer, 1987; Turchenek and Oades, 1979). In addition, it has been observed that the amount of organic matter per unit surface area is highest in the silt faction followed by the coarse clay fraction and lowest in the fine clay fraction (Broersa and Lavkulich, 1980; Kahle et al., 2002). The above findings are focused on particle size; however, there is little information on the relationship between mineralogy and the chemistry and bioavailability of soil organic matter (SOM).

In a study of the factors controlling C content across a range of New Zealand soils, no correlation was found between soil clay content and soil organic C ($r^2$ = 0.01) whereas a correlation was found between the soil organic C content and dissolved aluminum and allophone ($r^2$ = 0.57) (Percival et al., 2000). Furthermore, in seven soils from Germany containing from 14.9 to 21.2% clay content (dominated by illite, ~ 80 to 86%), the clay content explained 43% of the organic C content variability, moreover the cation exchange capacity + specific surface area of C free samples explained 90% of the organic C variability (Kahle et al., 2002). In another study it was reported that the organic C was preferentially sequestered in smectite rich sediments on continental slopes compared to those clay fractions dominated by chlorite (Ramson et al., 1998). In near-shore sediments, the organic matter was associated with "fine clays" composed mainly by phyllosilicates (illite/mica, Fe-rich chlorite, and minor amounts of kaolinite and vermiculite) (Bock and Mayer, 2000). By contrast, the amount of organic matter in the clay-size fraction was independent of the clay mineralogy in six kaolinite- and six smectite-dominated soils from around the world (Wattel-Koekkoek et al., 2001). However, in this study it was found that

kaolinite- and smectite-associated SOM are biochemically different. The kaolinite-associated SOM was enriched in polysaccharide products; whereas smectite-associated SOM was enriched in aromatic compounds as observed by pyrolysis-GC/MS and NMR. The above studies do not offer any evidence of the role of clay minerals on SOM dynamics and the formation of new humic substances.

The retention of $^{14}C$-labeled C increased with clay content among soils (4 to 34% clay content) incubated for 4-years with $^{14}C$-labeled cellulose (from barley straw) (Sorensen, 1981). When $^{14}C$-labeled-glucose, -hemicellulose, and -straw were added to a soil, it was found that the $^{14}C$ activity increased with clay content only for the $^{14}C$-labeled glucose and –hemicellulose amendments, whereas the $^{14}C$ specific activity decreased with clay content in the $^{14}C$-labeled hemicellulose and straw amendments (Christensen and Sorensen, 1985). The highest $^{14}C$ activity was associated with the clay fraction (<2 μm) after 5-6-year incubation, whereas the silt and sand fraction contained the lowest $^{14}C$ activity, and at the end of the experiment (18 years) the $^{14}C$ specific activity was similar in both clay and silt fractions (Christensen and Sorensen, 1985). In a different approach, Saggar and co-workers (1996, 1999) have reported that clay mineralogy rather that clay content influences mineralization and stabilization of microbial metabolites from $^{14}C$-labeled substrate. In a 35-day study, mineralization of $^{14}C$-labeled glucose was higher in soils with lower clay content and lower mineral surface area (Saggar et al., 1999). Similarly, in a 5-year study of $^{14}C$-labelled ryegrass amended soils, the $^{14}C$ activity was higher in soils with the highest surface area (vermiculitic and smectitic soils), whereas the lowest $^{14}C$ activity was found in low surface area soils (kaolinitic and

amorphous minerals) (Saggar et al., 1996).

The present study is a follow-up of work recently reported by Laird et al. (2001) and Laird (2001). Laird et al. (2001) investigated relationships between clay mineralogy and the chemistry of humic materials separated from the Ap horizon of a Webster-soil (Typic Endoaquoll). A relatively aggressive physical fractionation procedure was used to isolate the coarse (0.2-2.0 μm), medium (0.02-0.2 μm), and fine (<0.02 μm) clay fractions from the soil. The procedure separated mineralogically distinct fractions as the coarse, medium, and fine clay fractions were dominated by quartz, a low-charged interstratified illite/smectite phase, and smectite, respectively. While all of the fractions contained similar levels of organic C (52 to 70 g-C $kg^{-1}$ clay), the chemistry of the humic substances was substantially different among the clay fractions. The coarse clay fraction had a higher C:N ratio, stronger carboxyl and O-alkyl $^{13}C$-NMR peaks and lower levels of extractable amino acids, fatty acids, monosaccharides, and amino sugars than humics associated with the fine clay fraction. Analysis of the clay-humic complexes by scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (Laird 2001) revealed diffuse, filamentous films covering basal surfaces of 2:1 phyllosilicates in the medium and fine clay fractions and discrete particles (1 to 2 μm) of high-density metal-humic complexes in the coarse clay fraction. The results of Laird et al. (2001) and Laird (2001) indicate the existence of at least two distinct phases of mineral-associated SOM and suggest that clay mineralogy may contribute to the formation and/or stabilization of these materials. However, no evidence of the dynamics of new C additions to the clay-humic complexes was presented.

The samples used in the present study were from a previous study by Gale and Cambardella (2000). Gale and Cambardella (2000) grew oats (*Avena sativa L.*) in large pots under simulated no-tillage conditions and periodically pulse labeled the crop with $^{14}C$ by exposing the growing plants to $^{14}CO_2$. Several times during a 360-day post-harvest incubation, pots were destructively sampled and the level of $^{14}C$-activity in various aggregate size classes was quantified. Although the work was primarily focused on investigating mechanisms for aggregate formation and stabilization, the results also provide clear evidence for movement of new C from plant material through particulate organic matter (POM) and eventually into newly formed humic material associated with minerals in the silt + clay fraction. During the post-harvest incubation, 56.1% of the total $^{14}C$ in the pots on day one (day of harvest) was mineralized to $^{14}CO_2$. During the same period, $^{14}C$-activity associated with the coarse roots + large POM decreased from 74.2 to 9.7%, while $^{14}C$-activity associated with the clay + silt fraction increased from 17.1 to 23.9% of total $^{14}C$ in the pots on day one.

Availability of the $^{14}C$-labeled silt + clay fraction samples from the study of Gale and Cambardella (2000) provided us with an opportunity to further investigate the role of clay mineralogy in the stabilization of SOM. The specific objective of the study was to determine whether organic C in newly formed humic materials is uniformly distributed in the silt + clay fraction or is preferentially associated with either discrete C rich particles in the coarse clay fraction or the diffuse humic materials associated with surfaces of 2:1 phyllosilicates in the medium and fine clay fractions.

# MATERIAL AND METHODS

Gale and Cambardella (2000) grew oats in two growth chambers using large pots filled with Monona soil (fine silty, mixed, mesic Typic Hapludoll). The plants of one chamber were pulse labeled with $^{14}CO_2$ 15, 20, 26, 31, 37, 46, and 52 days after emergence. After senescence (118 days after emergence), the $^{14}C$-labeled and unlabeled plants were cut off at the soil surface, the residue was dried at 50 °C for 36 h and then returned to the pots. The $^{14}C$-labeled residue was placed on the soil surface of pots containing unlabeled roots and the unlabeled residues were placed on the soil surface of pots containing $^{14}C$-labeled roots. The pots were periodically sampled during 360 days of post-harvest incubation. During each sampling, the soil was separated into various aggregate size classes to study the relationship between the incorporation of new carbon into soil organic matter and the formation of soil aggregates (Gale and Cambardella, 2000). The finest fraction studied by Gale and Cambardella (2000) was the silt + clay fraction (<53 μm). To separate the silt + clay fraction, the soil was shaken for 18 h in a sodium hexametaphosphate solution and then the suspension was passed through a 53-μm sieve and rinsed with distilled water. The silt and clay fractions (material passed through the 53-μm sieve) was captured in a pan and dried at 70 °C for 36 h.

For the present study, the silt + clay fractions from the pots containing unlabeled surface residue and $^{14}C$-labeled roots and soil were dispersed in distilled water by shaking and the clay (<2 μm) and silt fractions were separated by sedimentation. The clay fraction was further separated into coarse (0.2 to 2.0 μm),

medium (0.02-0.2 μm), and fine (<0.02 μm) clay size fractions using a sonication-centrifugation-decantation technique (Laird et al., 1994). The various clay-humic fractions were Ca-saturated by washing in 0.5 M $CaCl_2$ four times, dialyzed against distilled water and freeze-dried.

The mineralogy of the various particle size fractions separated from the Monona soil was analyzed by X-ray diffraction (XRD). About 100 mg of Ca-saturated clay were slurried in 95% ethanol, oriented on glass slides by the paste method, and dried above a saturated solution of $Mg(NO_3)_2$ (54% relative humidity). Glycerol-solvated samples were prepared as above, except that glycerol was added to the ethanol. The oriented samples were analyzed between 2 and 32° 2θ with a Siemens D5000 x-ray difractometer using Cu Kα radiation. The $^{14}C$ activity was measured in each fraction by combusting the samples in a Harvey Biological Oxidizer, model OX500 (R. J. Harvey Instrument Corp., Hillsdale, NJ). The $^{14}C$-$CO_2$ released during oxidation was trapped in Harvey's $^{14}C$ cocktail and counted with the liquid scintillation analyzer. Organic C and total N content in each fraction was determined by dry combustion using a Carlo Erba NA1500 NSC elemental analyzer (Haake Buchler Instruments, Paterson, NJ). Elemental analysis (Al, Ca, Fe, K, Mg, Na and Si) of the clay samples was performed with an inductively coupled plasma-atomic emission spectrometer (ICP-AES) using the suspension nebulization technique (Laird et al., 1991a).

Scanning electron micrographs (SEM's) of the clay-humic complexes were obtained using a JEOL JSM-5800 LV Scanning Electron Microscope (JEOL U.S.A., Inc., Peabody, MA) operated at 10 KeV. To prepare the samples, 50 mg of the

various Ca-saturated samples were sonicated for 30 s at 40W in 15 mL of distilled water. Then, 50 μL of suspension were transferred to an aluminum-SEM stud covered with aluminum foil. The samples were dried in a desiccator and then sputter coated with gold-palladium.

## RESULTS

The XRD patterns of the Ca-saturated and glycerol-solvated clay fractions of the Monona soil are shown in Fig. 1 and Fig. 2, respectively. The fine clay fraction is dominated by interstratified smectite/illite, which is indicated by broad 14.5- (54% relative humidity) (Fig. 1) and 18.0-Å (glycerol solvated) (Fig. 2) XRD peaks. Further evidence for the interstratified smectite/illite includes the lack of a distinct 10-Å, illite XRD peak (Fig. 1 and Fig. 2) and the presence of relatively high amounts of K in the Ca-saturated fine clay fraction (Table 1). The nature of the interstratified smectite/illite in soils has been discusses previously (Laird and Nater, 1993; Laird et al., 1991b).

The XRD patterns for the coarse clay fraction show strong quartz, (4.3 Å) and weaker kaolinite (7.0 Å), illite (10 Å) and smectite/illite (14 Å) peaks (Fig. 1). Chemical analysis of the coarse clay fraction (Table 1) indicated higher concentrations of Si and K relative to the other soil clay fractions, which is consistent with higher levels of quartz and illite in the coarse clay fraction. The XRD patterns for the medium clay fraction were similar to those of the fine clay fraction. The high level of K and the lack of distinct 10 Å XRD peak indicate interstratified

smectite/illite. The silt fraction was dominated by quartz and feldspars.

Total C and total N content for the various fractions are presented in Table 2. Because no carbonates were present in this soil (Gale and Cambardella, 2000), the total C content is assumed to equal the organic C content. The organic C content in the clay fractions ranged from 30.6 to 39.3 g-C $kg^{-1}$-clay (medium and fine clay fraction, respectively). The total N content ranged from 3.4 g-N $kg^{-1}$-clay in the coarse clay fraction to 4.1 g-C $kg^{-1}$-clay in the fine clay fraction. Interestingly, the silt fraction contained much lower C and N content (4.45 and 0.50 g- $kg^{-1}$-silt, respectively). The C:N ratios were similar in all fractions and ranged from 8.5 in the medium clay fraction to 11.0 in the coarse clay fraction.

The specific activities of $^{14}C$ in the mineral fractions used in this study for three replicates (except for the fine clay fraction, which consisted of two replicates) are presented in Table 3. During the post-harvest incubation, $^{14}C$ specific activity (Bq $g^{-1}C$) increased from day-0 to day-360 in all particle size fractions (Table 3). Immediately after harvest (day 0), specific activity of $^{14}C$ ranged from 565.8 Bq $g^{-1}$-C in the coarse clay fraction to 847.2 Bq $g^{-1}$-C in the fine clay fraction; furthermore, there were no statistically significant differences between the specific activities of silt and coarse clay fractions and between the $^{14}C$ specific activities for the fine and the medium clay fractions ($P<0.05$). After 360 days of incubation, the specific activity ranged from 770.9 Bq $g^{-1}$-C in the coarse clay fraction to 1529 Bq $g^{-1}$-C in the fine clay fraction, and the $^{14}C$ specific activity was significantly different among mineral fractions ($P<0.05$). Surprisingly, the silt fraction had relative high $^{14}C$ specific activities for both day-0 and day-360 (526.6 and 1195, respectively). Thus during the

1 year post-harvest incubation the specific activity increased 108% in the silt fraction, 35% in the coarse clay fraction, 16% in the medium fraction, and 80% in the fine clay fraction.

Scanning electron micrographs for the various fractions are shown in Figures 3 to 10. The silt fraction (Fig. 3) contained numerous small (2 to 5 μm) mineral particles and fewer large (10 to 30 μm) particles. Some of the larger particles exhibited platy morphology while others appeared to be aggregates of numerous smaller particles. Also evident in the SEM's of the silt fraction are several organic particles (Fig. 4). The coarse and medium clay fractions were dominated by small (0.5 to 2 μm) discrete mineral particles (Figs. 5 and 6) but also contained a few larger aggregated particles (5 to 30 μm). The small discrete particles observed in the coarse clay fraction (Fig. 7) exhibited platy and roughly equal dimensional smooth rounded and smooth angular morphologies. By contrast the small particles in the medium clay fraction (Fig. 8) were dominated by wrinkled flakes. The fine clay fraction consists of large (20 to 100 μm) platy particles (Fig. 9). Surfaces of the large platy particles in the fine clay fraction are rough on a sub micron scale, with numerous valleys and ridges (Fig. 10).

## DISCUSSION

The $^{14}C$ specific activities found in the mineral fractions of the Monona soil (Table 3) provide direct evidence for the movement of new C from plant residue into the clay associated humic complexes. The relatively high $^{14}C$ specific activities in the

silt fraction are probably due to the presence of relatively undecomposed particulate organic matter in the silt fraction (Fig. 4) rather than to the presence of newly formed humic materials. Total organic C in the silt fraction was almost an order of magnitude lower than that found in the various clay fractions (Table 2), further suggesting that humic materials are not associated with the silt fraction. By contrast with the silt fraction, no evidence of particulate organic matter was observed in the SEMs of the various clay fractions. In previous work, Laird et al. (2001) and Laird (2001) found evidence of discrete high-density humic particles associated with the coarse clay fraction of a Webster soil (Typic Endoaquoll) and diffuse filamentous humic material coating surfaces of smectite and interstratified smectite/illite in medium and fine clay fractions of the Webster soil. The present study is consistent with the previous work, but provides additional evidence that newly formed humic materials preferentially accumulate in the medium and fine clay fractions relative to the coarse clay fraction. If C from the $^{14}C$-labeled roots had been randomly transformed into humic materials, then, all particle size fractions should have had similar specific activities. Furthermore, the organic C and the total N content are similar for coarse and fine clay fractions, yet the $^{14}C$ specific activities for the coarse clay fraction were roughly half that of the fine clay fraction (Table 3). Thus, the fine clay-associated humic substances may play an important role in the short-term organic matter turnover, whereas the coarse-clay associated organic matter is probably important in long-term organic matter cycling.

The separation of the clay fraction from the silt fraction by sedimentation gave, as expected from Stoke's Law, >2 μm uniform micro-aggregates (Fig. not

shown). In the fine clay fraction, the observed large platy particles (quasicrystals) (Fig. 9) do not exist in the soil. They were formed when smectites in the clay fraction were flocculated with $Ca^{2+}$ (Laird et al., 2001). In the coarse clay fraction (Fig. 5 and 7), quasicrystals are not formed nor expected since this fraction is dominated by quartz. However, some micro-aggregates survived the sonication-centrifugation steps of the fractionation (Fig. 5). Because of the morphology and because the XRD analysis indicated that the coarse clay is dominated by quartz (Fig. 7), these granular and round particles observed in the coarse clay are assumed to be quartz. On other hand, the platy particles observed in the coarse fraction are assumed either kaolinite or illite (Laird, 2001). From the results of SEMs, mineralogical and elemental analyses of the soil clay fractions, it is clear that the fine and coarse clay fractions have completely different mineralogy and morphology, as observed by other researchers (Laird, 2001; Laird et al., 2001).

Soil clay content has been used to determine whether clays influence soil organic C content and the chemical character of soil organic C. A poor correlation between clay content and total soil C in New Zealand grassland soils was found by Percival and co-workers (2000). Conversely, for two Australian soils, the soil with the higher clay content (54%) contained the higher organic C content (32 mg C $g^{-1}$ soil) and had a "more highly processed nature of carbon" (Baldock and Skjemstad., 2000). Whereas the soil with the lower clay content (11%) contained the lower C content (18 mg C $g^{-1}$) and a "greater plant-like chemical structure" of the organic C (Baldock and Skjemstad., 2000). Thus, clay content is only one of the variables influencing both soil organic C content and the chemical nature of SOM. Clay

mineralogy also plays an important role in the stabilization of soil organic C (Furukawa, 2000; Laird et al., 2001; Ramson et al., 1998; Saggar et al., 1999; Saggar et al., 1996). Soil organic C appears to be preferentially sequestered in smectite-rich continental sediments of California and Washington (Ramson et al., 1998). By using electron energy-loss spectroscopy, it was observed that the organic matter within clay aggregates co-exists with calcium, suggesting that Ca-smectites, rather than Ca-illite or Ca-kaolinite are preferentially associated with the organic matter of aquatic sediments from the Jourdan River (Furukawa, 2000). In our study, it is apparent from the $^{14}C$ specific activity and the mineralogy data that newly formed humic substances either accumulate on or are formed on smectite surfaces.

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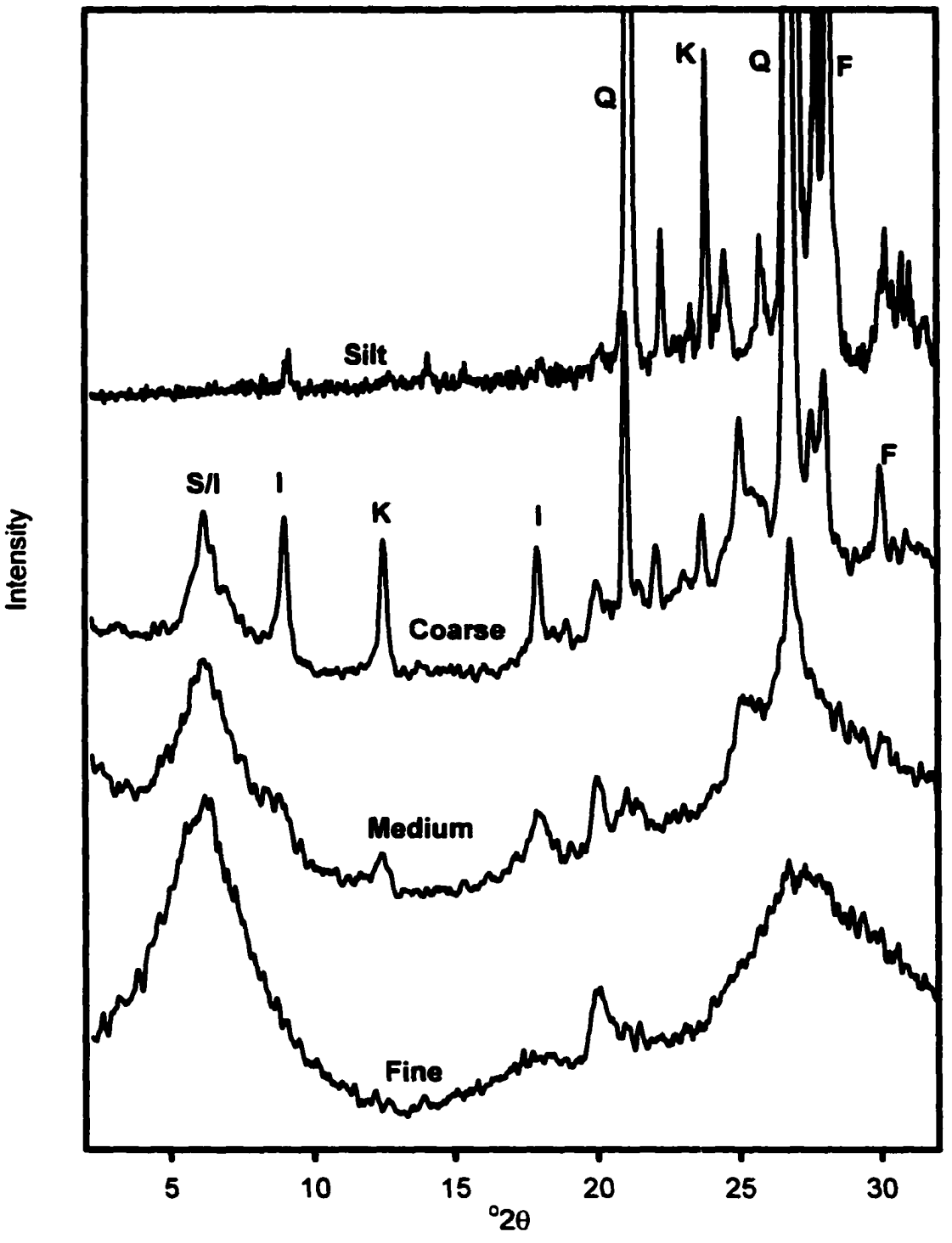



Fig. 1. XRD patterns of silt and clay fractions from a Monona soil (S/I: smectite/illite; I: illite; K: kaolinite; Q: quartz; F: feldspars).

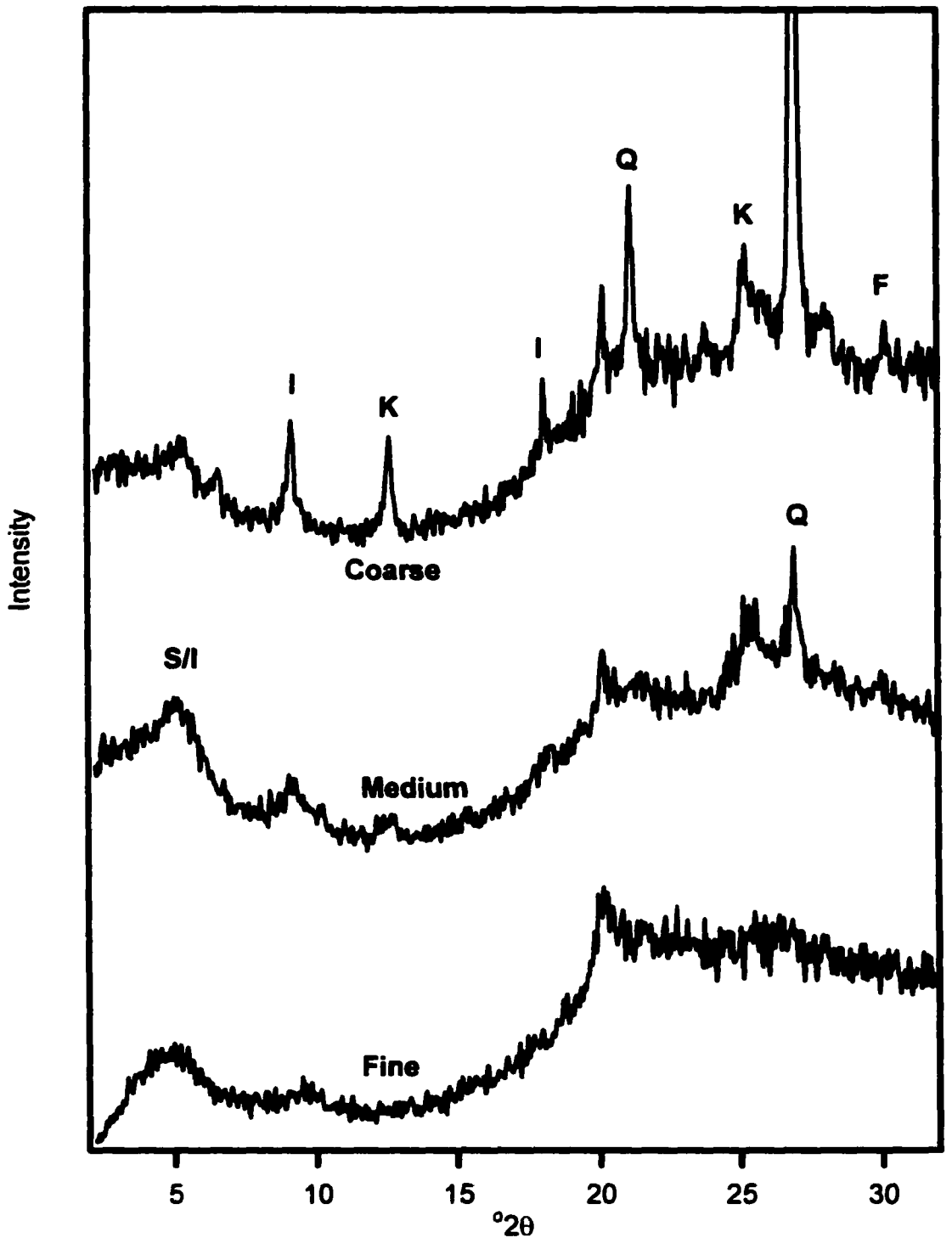



Fig. 2. XRD patterns of glycerol-solvated clay fractions from a Monona soil (S/I: smectite/illite; I: illite; K: kaolinite; Q: quartz; F: feldspars).

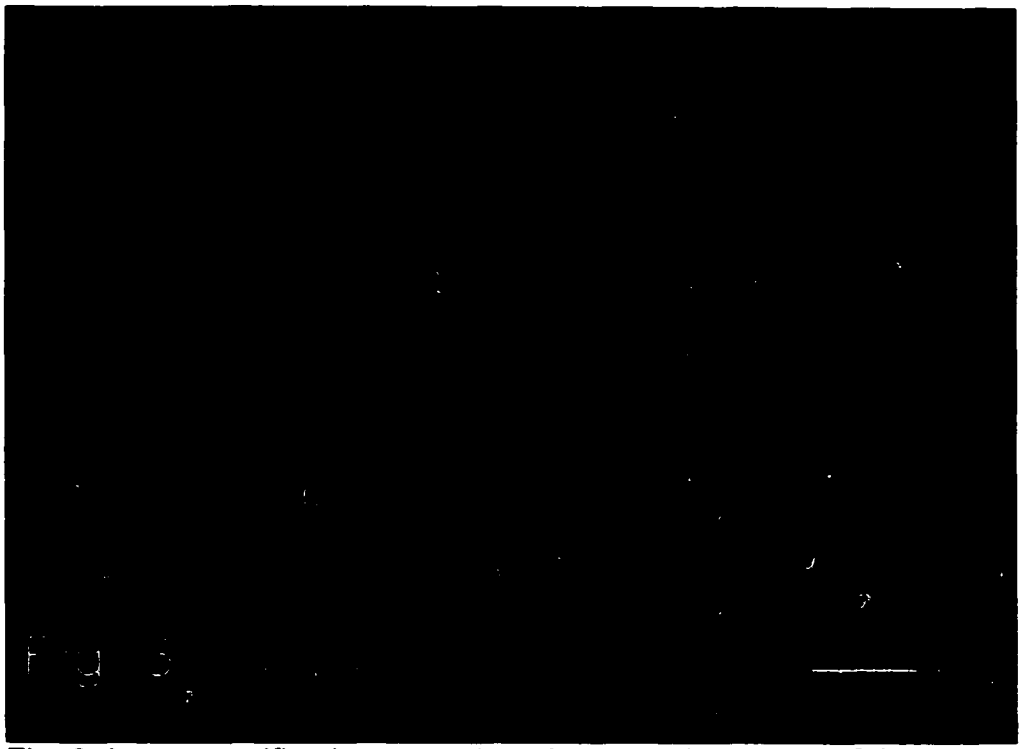


Fig. 3. Low magnification-scanning electron micrograph of the silt fraction from a Monona soil.

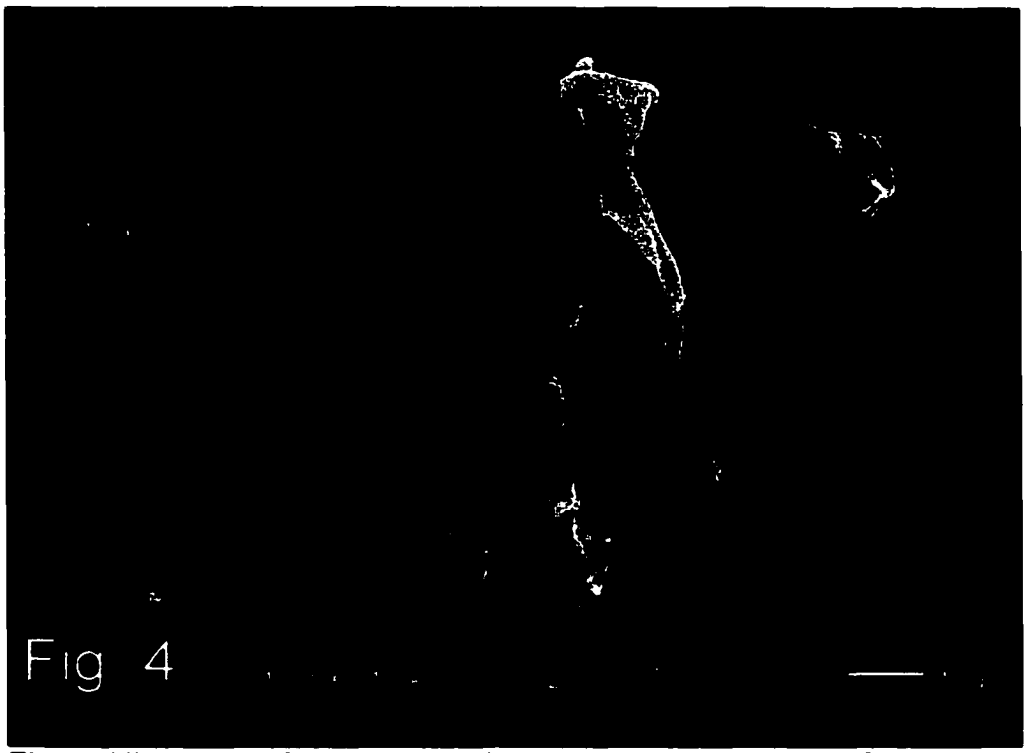



Fig. 4. High magnification-scanning electron micrograph of the silt fraction from a Monona soil.

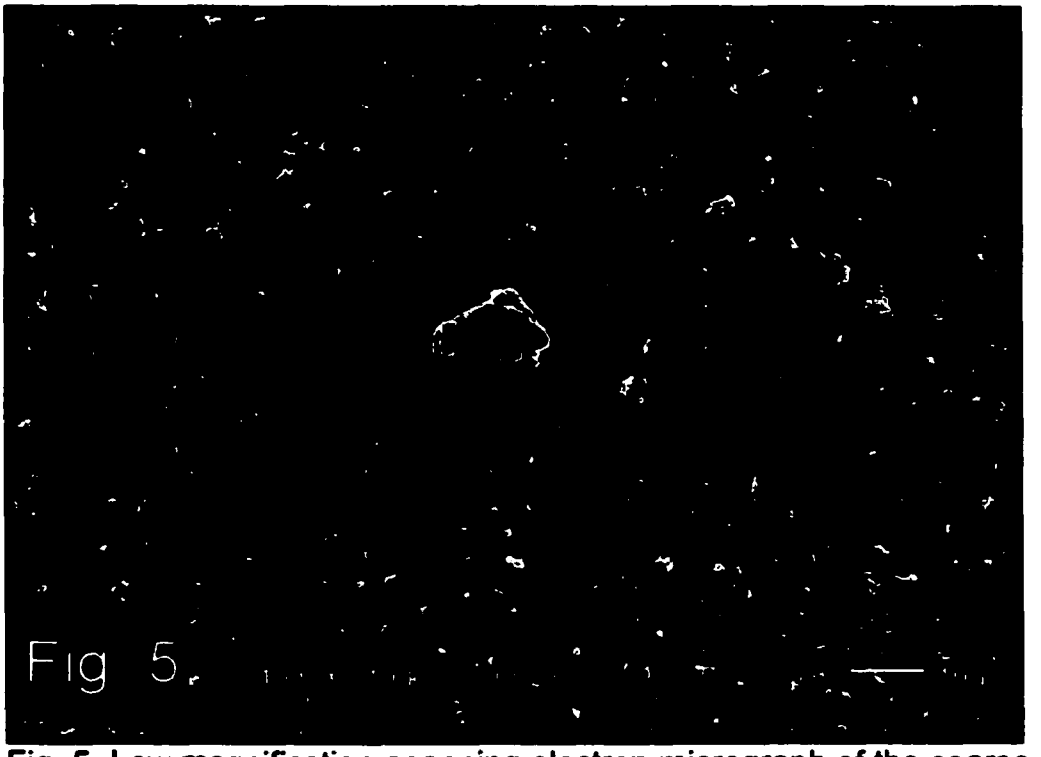



Fig. 5. Low magnification-scanning electron micrograph of the coarse clay fraction from a Monona soil.


Fig. 6. Low magnification-scanning electron micrograph of the medium clay fraction from a Monona soil.


Fig. 7. High magnification-scanning electron micrograph of the coarse clay fraction from a Monona soil.


Fig. 8. High magnification-scanning electron micrograph of the medium clay fraction from a Monona soil.


Fig. 9. Low magnification-scanning electron micrograph of the fine clay fraction from a Monona soil.

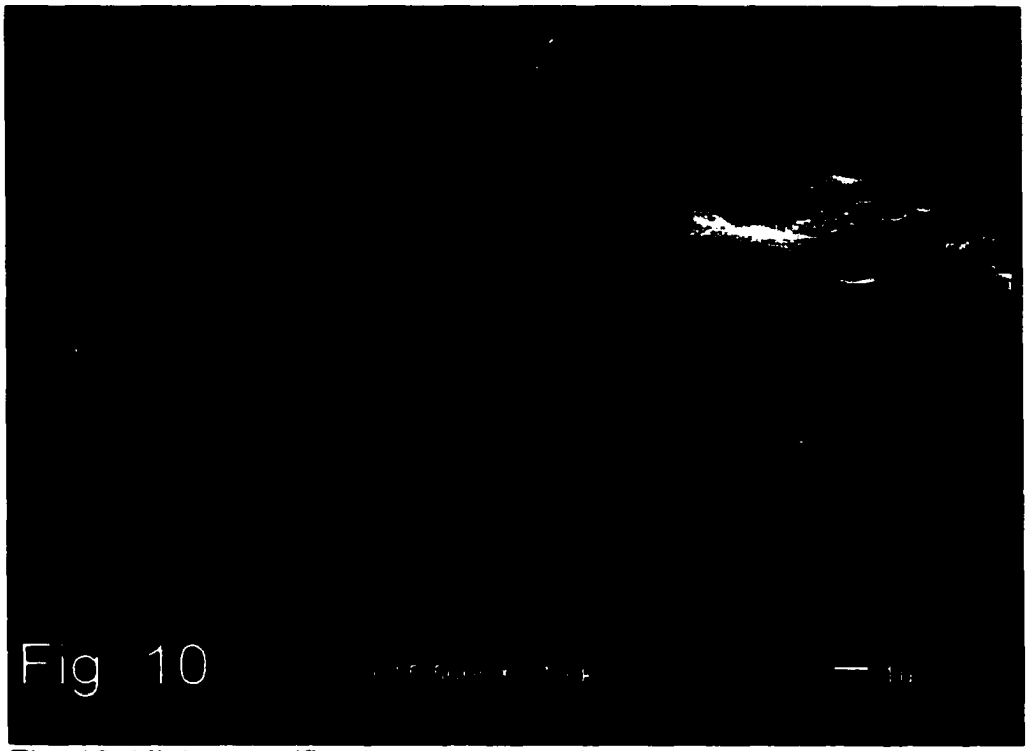



Fig. 10. High magnification-scanning electron micrograph of the fine clay fraction from a Monona soil.

Table 1. Chemical composition of soil clay fractions from the Monona Soil.

| Element | Soil Clay Fraction | | |
|---|---|---|---|
| | Coarse | Medium | Fine |
| | *g kg$^{-1}$ oxides* | | |
| Si | 294 | 258 | 270 |
| Al | 98.1 | 133 | 124 |
| Fe | 65.9 | 76.8 | 83..4 |
| Mg | 12.0 | 15.0 | 13.2 |
| Ca | 9.11 | 14.4 | 20.1 |
| Na | 4.63 | 1.00 | 0.45 |
| K | 30.4 | 24.3 | 11.5 |

Table 2. Chemical properties of silt and clay fractions from a Monona Soil.

| Element | Soil Fraction | | | |
|---|---|---|---|---|
| | Silt | Coarse Clay | Medium Clay | Fine Clay |
| | ——— g $kg^{-1}$ ——— | | | |
| Organic C | 4.45 a† | 37.0 b | 30.6 c | 39.3 d |
| Total N | 0.50 a | 3.4 b | 3.6 c | 4.1 d |
| C:N ratio | 9.9 | 11 | 8.5 | 9.6 |

† Within each element, means followed by the same letter are not significantly different by the Student-Newman-Keuls test (P<0.05).

Table 3. Specific activity of $^{14}C$ in the mineral fractions from the Monona Soil.

| Day | Soil Fraction | | | |
|---|---|---|---|---|
| | Silt | Coarse Clay | Medium Clay | Fine Clay |
| | *Bq g$^{-1}$* | | | |
| 0 | 526.6 a† | 565.8 a | 733.3 b | 847.2 c |
| 360 | 1195 b | 770.9 a | 855.0 a | 1529 c |

† Within the same sampling day, means followed by the same letter are not significantly different by the Student-Newman-Keuls test ($P<0.05$). Three replicates for each fraction, except the fine clay fraction, which consisted of two replicates.

# CHAPTER 3

# CATALYTIC CONDENSATION OF GLUCOSE BY CLAY MINERALS

A paper to be submitted to Clays and Clay Minerals

Javier M. Gonzalez and David A. Laird

## ABSTRACT

Soil organic matter (SOM) has important functions in soils. A better understanding of SOM formation and humification is necessary to develop suitable soil management practices for the conservation of SOM. The general objective of this study was to determine whether smectites and synthetic goethites (with different degree of Al-substitution) abiotically catalyze glucose polymerization to form humic-like substances. Four cation-saturated smectites and four Al-substituted goethites were abiotically incubated with glucose solutions for 21 days at 37°C. The smectites were classified as montmorillonite, beidellite, saponite and "ferruginous smectite". Al-substitution in synthetic goethites was 0, 2.0, 5.1 and 9.4% mol/mol Al/(Al+Fe). After the incubations, glucose recoveries in the solutions ranged from 18.3 to 98.3% and from 58.3 to 83.2% for smectites and goethites, respectively. However, soluble organic C recoveries ranged from 95 to 109.3% and from 106.0 to 110.1% for

smectite and goethites systems, respectively, relative to the amount of C added as glucose. Higher glucose recoveries for "Fe-rich" smectites were obtained, whereas lower glucose recoveries were obtained for "Fe-poor" smectites. The results suggest that smectites abiotically catalyze glucose dehydration to form furfural compounds and levulinic acid (4-oxopentanoic acid) under conditions similar to those found in soils. Abiotic polymerization of furfural compounds and levulinic acid may be a major pathway leading to formation of humic materials in soils.

## INTRODUCTION

Soil organic matter (SOM), which typically ranges from less than 1% (sandy soils) to up 10% (poorly drained soils) of the mass of a soil (Stevenson, 1982), plays many important functions. From an agricultural point of view, SOM affects the physical, chemical and biological properties of soils. SOM significantly improves stabilization of the soil structure, and thus increases aeration, water-holding capacity and permeability of soils. SOM also indirectly increases nutrient availability to plants via mineralization of organic forms of N, P, S and other nutrients. From an environmental point of view, SOM reduces transport of organic and inorganic contaminants to water (groundwater and surface water) by binding those contaminants to its network. Furthermore, the sequestration of C in SOM has been suggested as one means of reducing the rate of increase of greenhouse gases in the atmosphere.

SOM can be fractionated into humic and nonhumic substances. Humic substances, which are a diverse group of relatively high-molecular-weight compounds formed from secondary synthesis reactions, can be fractionated by their solubility in

alkaline/acidic solutions into fulvic acid, humic acid and humin. Nonhumic substances consist of well-known classes of biochemical compounds such as proteins, carbohydrates, and lipids.

The decay of plant and animal material in soil is a multi-stage process involving many different organisms. Macrofauna reduce the size of plant and animal material, whereas microorganisms support enzyme-catalyzed reactions that transform the particulate organic matter into small molecules such as proteins, peptides, amino acids, carbohydrates, and fatty acids.

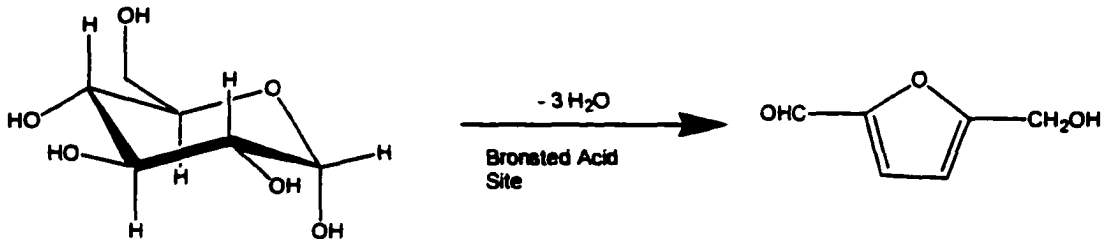
Soil carbohydrate content (mainly as polysaccharides) accounts for 10% of the SOM (Cheshire, 1979). Neutral sugars account for 59% (27% glucose) and acidic sugars account for 29% (14% as glucuronic acid) of the soil carbohydrate composition (Cheshire, 1979). Soil carbohydrates have different functions; they, (a) act as binding agents of soil particles to form stable aggregates, (b) help promote the retention of soil water, (c) contribute to the cation exchange capacity, (d) contribute to the complexation of metals and organic pollutants, and (e) serve as a C and energy source for soil organisms (Arfaioli et al., 1997; Cheshire, 1979; Koivula and Hanninen, 2001; Stevenson, 1982; Stevenson and Cole, 1999).

It has been suggested that carbohydrates are precursors of humic acids, however, this theory is not as widely accepted as the lignin degradation hypothesis (Koivula and Hanninen, 2001). The accumulation of humified SOM in soils may be enhanced by the formation of carbohydrate-metal complex (Cu, Al, Zn, Fe), which protect the carbohydrates from microbial degradation (Martin et al., 1966; Martin et al., 1972). Furthermore, the condensation of amino acids and sugars to form melanoidins,

through the Maillard reaction, has been hypothesized as one pathway for the synthesis of humic substances (Stevenson, 1982). Infrared spectra of "pseudomelanoidin" formed from galactose (under hot alkaline conditions) showed the presence of aromatic (furanoid), carbonyl and hydroxyl groups (Rubinsztain et al., 1986), suggesting that these compounds could be building blocks of humic substances (Ikan et al., 1986). By contrast, melanoidins formed from glycine-galactose contained few if any of these moieties (Rubinsztain et al., 1986).

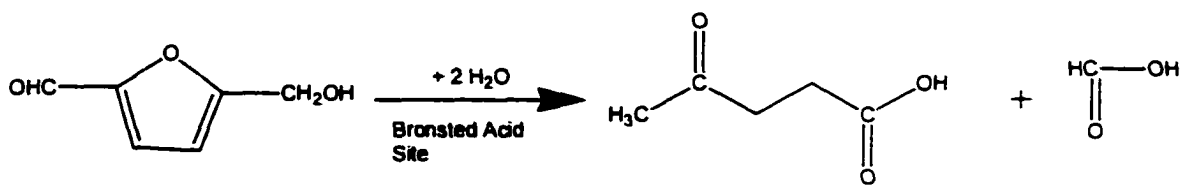
Carbohydrates undergo various acid-base catalyzed reactions including; mutarotation, enolization, and $\beta$ elimination. Mutarotation is the inter-conversion between $\alpha$ and $\beta$ anomers and between cyclic and open chain forms of sugars. Glucose in solution exists in equilibrium as both pyranose (cyclic structure) and open chain forms (Bunn and Higgins, 1981). Under acidic conditions, >99% of glucose is in the form pyranose (both $\alpha$ and $\beta$ forms) (Bunn and Higgins, 1981). Enolization, an attack on the $\alpha$-carbon to form an unsaturated alcohol, is followed by $\beta$ elimination of a hydroxyl or an alkoxyl group from the $\beta$-carbon atom (Pigman and Anet, 1972). Under alkaline conditions, $\beta$ elimination is the rate-limiting step that leads to the formation of saccharinic acids (Pigman and Anet, 1972). Under acidic conditions, enolization is the rate-limiting step and $\beta$ elimination leads to the formation of furfural compounds (Pigman and Anet, 1972).

Furfural compounds are intermediate compounds for the most advanced stages of the Maillard reaction (Ferrer et al., 2000). Glucose dehydration occurs under acidic conditions to form 5-(hydroxymethyl)-2-furfural (HMF) (Lourvanij and Rorrer, 1994; Lourvanij and Rorrer, 1997).




Glucose HMF

HMF has three reactive groups; the hydroxymethyl group, the aldehyde group and the furan ring. The hydroxymethyl group behaves as a primary alcohol; thus, ester formation and oxidation of this group may occur. The aldehyde group is attached to an aromatic moiety; however, contrary to the aromatic aldehyde that forms furoin derivatives or hydrofuramide when reacted with ammonia; this functional group forms "resinous" material (Newth, 1951). The most important aspects of the chemistry of the furan ring are hydrogenation and its cleavage. Aqueous HMF was completely transformed to unknown compounds after 186-day at room temperature (Lehnen et al., 2001). In aqueous conditions, the cleavage of the furan ring is acid-catalyzed to yield levulinic acid (4-oxopentanoic acid) and formic acid according to the following reaction (Lourvanij and Rorrer, 1994; Lourvanij and Rorrer, 1997; Newth, 1951):




HMF Levulinic acid Formic acid

Soil clay minerals, predominantly smectites and oxyhydroxides of Al and Fe, are very important in SOM stabilization and humification because of their chemical properties. Smectites have large surface areas and high cation exchange capacities and are further classified as montmorillonite (dioctahedral, Mg-Al octahedral substitution), beidellite (dioctahedral, Al-Si tetrahedral substitution), nontronite (Fe-rich, dioctahedral, Al-Si tetrahedral substitution), hectorite (Mg-rich, trioctahedral, Li-Mg octahedral substitution), saponite (Mg-rich, trioctahedral, Al-Si tetrahedral substitution), and sauconite (Zn-rich, dioctahedral, Al-Si tetrahedral substitution) (Reid-Soukup and Ulery, 2002). Smectites behave as Lewis acids when incompletely coordinated Al and Fe ions are exposed at the lateral edges or when interlayer cations are dehydrated (Heller-Kallai, 2002). When hydrated, exposed non-bridging OH groups tetrahedrally or octahedrally coordinated with Si, Al, and Fe are weak Bronsted acids (Heller-Kallai, 2002). In hydrated systems the acidic character of smectites increases with the polarizing power of the exchangeable cations (decreasing size and increasing charge). Furthermore, as the number of water molecules coordinated to the exchangeable cations decreases, the remaining water molecules become increasingly polarized and are thus better able to donate protons (Heller-Kallai, 2002).

Iron oxides, hydroxides, and oxyhydroxydes (named collectively "Fe oxides" in this dissertation) are widespread components of soils. Goethite ($\alpha$-FeOOH), the most common Fe oxide in soils, consists of double chains of Fe atoms octahedrally coordinated to O and OH. The doubled chains are bound to neighboring double chains by Fe-O-Fe and H bonds and separated by double chains of vacant sites that

form tunnels (Bigham et al., 2002; Schwertmann and Taylor, 1989). Al-substitution is common in Fe oxides, particularly goethite (Schwertmann, 1988). Fe oxides in soils have a pH dependent or variable surface charge because of the weakly acidic non-bridging terminal FeOH groups (Schwertmann and Cornell, 1996). At soil pH's, many Fe oxides are positively charged, and therefore readily react with negatively charged groups on humic substances. $^{13}C$ NMR has shown strong peaks for carboxyl groups in oxide-associated organic matter (Oades et al., 1989) and FT-IR studies have shown that carboxyl groups react with FeOH groups by ligand exchange (Huang, 1995). Fe oxides have little effect on the polymerization of phenols (Shindo and Huang, 1984). On the other hand, the mineralization of amino acids was catalyzed in the presence of iron oxides, but inhibited when phenols were present, suggesting that Fe oxides catalyze the condensation of phenols and amino acids (Wang, 1995).

Because of surface acidity, smectites can catalyze numerous organic reactions including: Diels-Alder cycloadditions (Heller-Kallai, 2002; Laszlo, 1987), Friedel-Crafts reactions (Heller-Kallai, 2002; Laszlo, 1987), aldol reactions (Izumi, 1992), Michael additions reactions (Izumi, 1992), and oxidation reactions (Heller-Kallai, 2002). Furthermore, there is evidence that pillared montmorillonite facilitated four acid-catalyzed reactions involving sugars: isomerization of glucose to fructose, dehydration of glucose to HMF, cleavage of HMF to formic acid and levulinic acid and formation of solid and water soluble humic-like substances (Lourvanij and Rorrer, 1994; Lourvanij and Rorrer, 1997). Also, these researchers reported that the rate constants of isomerization and dehydration of glucose were lower than the rate constants of levulinic acid and formic acid formation. Furthermore, they indicated that

the formation of humic-like substances was higher with levulinic acid compared with that from formic acid. Thus, it is hypothesized that smectites catalyze the formation of condensation products from glucose under conditions relevant to the formation of humic materials in soils. There is little of information on the role of Fe oxides in the transformation of glucose under soil conditions.

The specific objectives of this study were: (1) to determine whether smectites (type of smectite) and their associated saturating metal cations catalyze the abiotic polymerization of glucose to form humic-like compounds and (2) to determine if synthetic Al-substituted goethites can catalyze the formation of humic-like compounds from glucose.

## MATERIALS AND METHODS

### Chemicals

Chemicals: ACS reagent grade D-glucose (Sigma-Aldrich Corp., St. Louis, MO) was used without further purification. DABS-hydrazine, synthesized from DABS-chloride [4-(Dimethylamino)azobenzene-4-sulfonyl chloride] (96% purity, Sigma-Aldrich Group, St. Louis, MO), was used for the pre-column derivatization of glucose. Milli-Q water (Milli-Q system, Millipore, Bedford, MA) with a resistivity of 18.2 MΩ-cm was used to prepare all solutions.

### Samples

Four different reference smectites with a range of surface properties were used in this study. Panther Creek beidellite (Panther) was obtained from the A.D. Scott

collection, Iowa State University, Ames, IA. Otay white montmorillonite (Otay) was collected at an exposure near San Diego, CA. IMV saponite (Saponite) was obtained from IMV Cooperation, a division of Floridin Company (Amargosa Valley, NV). Ferruginous Smectite SWa-1 (SWa) was obtained from the Source Clay Repository of the Clay Minerals Society (Columbia, MO). For each sample, the clay fraction (<2 μm) was separated from the bulk ore by sedimentation. The clays were Na-, Ca-, Cu-, and Al-saturated by washing the <2 μm clays twice with 1 M solutions and twice with 0.1 M solutions of the appropriate salts (NaCl, $CaCl_2$, $CuCl_2$, and $Al(NO_3)_3$). Excess salt was removed by dialyzing the $M^{n+}$-saturated clays (where $M^{n+}$ is the saturating cation) using Spectra/Por® 3500 MWCO molecular porous membrane tubing (Spectrum Laboratories Inc., Rancho Dominguez, CA) against Milli-Q water until the conductivity of dialysate was <0.6 mS/m. The $M^{n+}$-saturated clay samples were freeze-dried and saved for later use.

Portions of the Na-clays were coated with polymeric $Fe(OH)_3$ (Fusi et al., 1989). Briefly, polymeric $Fe(OH)_3$ was prepared by dialyzing 0.1 M $Fe(NO_3)_3$ against water for 6 h, using a 3500 MWCO molecular porous membrane tubing. Then, 25 mL of polymeric $Fe(OH)_3$ was added to a Na-saturated clay suspension (5 g clay + 150 mL water) to prepare the $Fe(OH)_3$-coated clays (Fe-coated clays), mixed for 1 h, washed and centrifuged until the conductivity of supernatant was <0.6 mS/m. The Fe-coated clay samples were freeze-dried and saved for later use.

Four Al-substituted goethites with different Al/(Fe+Al) mol/mol ratios were synthesized (Schwertmann and Cornell, 2000). Briefly, an aluminate solution was prepared by mixing 500 mL of 0.5 M $Al(NO_3)_3$ solution and 300 mL of 5.0 M KOH in

polyethylene containers. Then, 0, 20, 50 and 120 mL of 0.3125 M aluminate solution and 180, 178, 174, and 165 mL, respectively, of 5.0 M KOH were mixed in polyethylene containers. Next, 100 mL of freshly prepared 1.0 M $Fe(NO_3)_3$ solution was added quickly to each container, the samples were diluted to 2 L with distilled water, mixed and placed in the oven for 14 days at 70°C. During the incubation, the samples were shaken once a day. After crystallization, the samples were centrifuged to separated the precipitated, and the precipitate was washed twice with 400 mL 1.0 M KOH to remove excess Al, then the pH was adjusted to 7.5 with HCl, washed once with distilled water, and freeze dried.

**Incubations**

About 0.25 or 0.50 g of $M^{n+}$-saturated clays or Al-substituted goethites were placed in 15-mL amber vials and loosely capped using PTFE/silicone septa (Supelco, Bellefonte, PA). The capped vials were sterilized in an autoclave for 15 min at 120 °C. Glucose solutions were prepared fresh for each experiment, filter-sterilized with 150-mL Nalgene® MF75 Series sterile disposable tissue culture filter units (Nalge Nunc International, Rochester, NY), and added to the cooled-vials containing the sterilized clay or goethite under sterile conditions to avoid microbial contamination. The solutions were dispensed using a Repeater® Plus pipet with autoclave-sterilized 50-mL Eppendorf Combitip Plus tips (Eppendorf AG, Hamburg, Germany). The final concentration of glucose was about 1.0 mmol $g^{-1}$-clay or goethite and the total solution volume was 5 mL. The vials containing the $M^{n+}$-saturated clay (or goethite) + glucose systems were capped tightly and a strip of wax paper was wrapped around the caps

to further insure the integrity of the systems. The vials were vortex-mixed and incubated in the dark for 21 days in a temperature-controlled incubator at 37±0.5 °C. Three replicates were run for each treatment.

**Analysis**

After incubation, the evolved carbon dioxide was measured with a CI-301 $CO_2$ infrared gas analyzer (CID, Inc., Vancouver, WA). $CO_2$ concentrations in the vial's headspace were determined by circulating He gas through a closed loop connected to the infrared analyzer. Standard curves were constructed for $CO_2$ by injecting a range of known concentrations of $CO_2$ into the infrared gas analyzer. Certified standard $CO_2$ (10% in He) was obtained from Scott Speciality Gases (Troy, MI).

After the $CO_2$ analysis, 5-mL of a filter-sterilized 100 mM $CaCl_2$ solution were added to the vials under sterile conditions, mixed and centrifuged for 10 min at 4500 x *g*. The supernatant was analyzed for glucose and soluble organic C, and the pH of the supernatant was measured.

Glucose analysis was performed by reverse phase HPLC using a pre-column derivatization method with DABS-hydrazine (Muramoto et al., 1987) to form a chromophore. The DABS-hydrazine was synthesized in our laboratory following the procedure of Muramoto and co-workers (1987). Briefly, a solution of DABS-Cl in chloroform (500 mg/60 mL) was added dropwise with stirring to a solution of 0.62 mL of hydrazine hydrate in 60 mL of methanol at room temperature. The mixture was stirred for 30 min at room temperature and then concentrated using a rotary evaporator. The precipitate was washed with chloroform and dried to yield 370 mg of

DABS-hydrazine. A DABS-hydrazine solution (0.1%, w/v) was prepared by dissolving 10 mg of DABS-hydrazine in 10 mL of 100% ethanol, 250 μL of aqueous trichloroacetic acid (10%) was added, the solution was mixed, and then stored at 4°C.

For the glucose analysis, a 25-μL aliquot of supernatant from one of the incubated clay + glucose samples was placed in a 2-mL clear vial and dried under a stream of $N_2$ gas in a sand bath at 40 °C. Then, 400-μL of DABS-hydrazine solution was added to the clay sample; the vials were capped and reacted for 30 min at 70 °C. The solution was allowed to cool at room temperature and immediately analyzed by reverse phase-HPLC. The analysis was performed by using a HP 1050 quaternary pump and an 1100 diode array detector (Hewlett-Packard, Wilmington, DE). A reverse phase ODS $C_{18}$ column (4.6 by 150 mm, 5 μm particle size) (Supelco, Bellefonte, PA) was used. The injection was performed using a 10-μL loop Rheodyne 7125 syringe loading injector (Rohnert Park, CA), and absorbance was measured at 470 nm. The mobile phases consisted of acetonitrile (A) and a mixture of 40% acetonitrile/60% Milli-Q water adjusted at pH 3.0 with HPLC-grade $H_3PO_3$ (B). A flow rate of 0.8 mL $min^{-1}$ was used with the following gradient profile: 0 min 100% B; 6.0 min 100% B; 10 min 10% B; 15 min 10% B, with a re-equilibration time of 5 min. The acetonitrile/water mixture of the mobile phase (B) was used to minimize the unstable pressure in the HPLC pump when water and acetonitrile are mixed. The retention time for glucose was 5.6 min. Calibration was performed using external standards. Peaks for the un-reacted DABS-Cl and DABS-hydrazine were observed at 3.0 and 14 min, respectively. The detection limit for this method is 2 pmol (Muramoto et al., 1987).

Organic carbon in the supernatant was determined using a modified potassium

dichromate oxidation method (Yeomans and Bremner, 1988). An aliquot of 2.0 mL of supernatant, 5.0 mL of 1 N $K_2Cr_2O_7$ and 7.5 mL of concentrated $H_2SO_4$ were added to a 125-mL Erlenmeyer flask with a TS Joint. The flask was placed on a hot plate and connected to a Liebig condenser for 15 minutes. The temperature was controlled in order to have a stable boiling of the solution. The flasks were allowed to cool to room temperature, 0.3 mL of indicator solution (0.1 g of *N*-phenylanthranilic acid and 0.1 g $Na_2CO_3$ dissolved in 100 mL of Milli-Q water) was added, and the samples were titrated with Mohr's salt solution. The end point is evident by a bright violet color. The Mohr's salt solution consisted of 156.8 g of ferrous ammonium sulfate [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_20$] dissolved in 100 mL concentrated sulfuric acid and then taken to 2.0-L volume with Milli-Q water and kept in an amber bottle. This solution was standardized daily because it undergoes slow oxidation during storage. Two boiled controls and two unboiled controls were also prepared.

X-ray diffraction (XRD) patterns for the clay minerals and oxides in this study were obtained using a Scintag XDS-2000 powder diffractometer (Thermo ARL, Ecublens, Switzerland) equipped with an air-cooled Kevex Psi Peltier Silicon detector, and CuK$\alpha$ radiation. About 100 mg of $M^{n+}$-saturated clay were slurried in 95% ethanol, oriented on glass slides by the paste method and dried above a saturated solution of $Mg(NO_3)_2$ (54% R.H.). Data were collected from 2 to 32° and 2 to 72° 2$\theta$, for the clay minerals and goethites, respectively. Infrared spectra were acquired for the freeze-dried samples using the Diffuse Reflectance Infrared Fourier Transformed (DRIFT) technique in a Nicolet model Magna 500 equipped with a DTGS detector (Madison, WI). About 5 mg of sample and 95 mg of FT-IR grade KBr were ground in

a mortar and placed in small cup sample holder. A total of 64 individual scans were signal-averaged with an optical resolution of 4 $cm^{-1}$. Elemental analysis of the clay samples was performed by inductively coupled plasma-atomic emission spectroscopy using the suspension nebulization technique (Laird et al., 1991). Furfural compounds were identified by reverse phase-HPLC (Ferrer et al., 2000) using the same HPLC system as in glucose analysis. An aliquot from the supernatant of the samples was filtered through a 0.02 µm pore-size filter and injected using a 10µL injection loop. The mobile phases consisted of 5:95% acetonitrile:water (A) and acetonitrile (B). A flow rate of 0.85 mL $min^{-1}$ was used with the following gradient profile: 0 min 100% A; 8.0 min 100% A; 10 min 50% A; 14 min 50% A, with a re-equilibration time of 5 min. The absorbance was measured at 284 nm. The retention times for levulinic acid, 5-(hydroxymethyl)-2-furfural, furfural, and methylfurfural were 2.0, 5.6, 6.8, and 11.8 min, respectively.

One Way Analysis of Variance (ANOVA) and pairwise multiple comparison procedures using the Student-Newman-Keuls Method ($P<0.05$) were performed using SigmaStat software (SPSS Science, Chicago, IL).

## RESULTS

### Characterization of clay minerals and Fe oxides

The four clay minerals used in this study were chosen because of their chemical differences. The elemental analysis for all clays is shown in Table 1. Also, the $Fe^{3+}$ content in the Fe-coated clays is shown. Typical XRD patterns for the smectites are shown in Fig. 1. The XRD analysis of the Ca-saturated clay minerals,

equilibrated at 54% R.H., shows a large peak from 1.37 to 1.56 nm. In addition to the smectites peaks, the Saponite XRD pattern has two small peaks with *d*-spacing of 1.02 and 0.33 nm, indicating the presence of illite and quartz, respectively. A relative broad and asymmetrical peak near 0.44 nm for Otay and SWa clays indicates relatively poor orientation of the clays when the samples where prepared for the XRD analysis. The total C and N content in the clays used in this study ranged from 0 to 0.39% and from 0 to 0.02%, respectively (Table 1). FT-IR spectra for all four smectites (Fig. 3) show broad OH stretching bands of structural hydroxyl groups from 3300 to 3600 $cm^{-1}$, a sharp OH deformation band of water about 1625 $cm^{-1}$, and Si-O stretching modes near 1000 $cm^{-1}$. For the Otay clay, a sharp Si-O stretching (longitudinal mode) band is observed about 1100 $cm^{-1}$ (Madejova and Komadel, 2001). Saponite shows an additional band about 1470 $cm^{-1}$, corresponding to the C-O stretching of $CO_3^{2-}$, explaining the relative high C content (0.39%) in this clay (Table 1). The interlayer charge (from 0.38 to 0.58), determined from the sum of $Ca^{+2}$, $K^{+}$ and $Na^{+}$ cations, is within the range for smectites (Table 1).

Al-substituted goethites can be differentiated by elemental analysis, XRD patterns, and FT-IR spectra. The elemental analysis for all four goethites is presented in Table 2. The Al-substituted goethites were named Goe-00, Goe-20, Goe-51, and Goe-94 after their Al-substitution (0, 2.0, 5.1, and 9.4% mol/mol Al /(Al+Fe), respectively). The total C content ranged from 0.10 to 0.15%, whereas total N content was negligible. The XRD patterns of the four Al-substituted goethites are presented in Fig. 2. All four samples showed typical goethite reflections *(hkl)*:*110, 120, 130, 111, 140, 021, 101, 040* and *121*. FT-IR spectra for all four Al-substituted goethites (Fig.

4) show that the Fe-O-H deformation band for Goe-00, -20, -51, and -94 were 892, 902, 905, and 911 $cm^{-1}$, respectively.

**Total C in solution**

The mass balances of C for the $M^{+n}$-clay + glucose and Al-substituted goethite + glucose systems are presented in Tables 3a, 3b, and 4. The total C recovered for clay mineral systems ranged from 95.0 to 109.6%. The effect of clay type and the effect of saturating cation on the total soluble C recovered in clay systems are presented in Tables 3b and 3a, respectively. Generally, the soluble organic C content was significantly different among saturating cations; Fe > Ca = Na > Al > Cu. The total C recovered for goethites ranged from 106.0 to 110.1% (Table 4). $CO_2$ evolved from both systems were negligible, 0.0 to 0.2%.

**Glucose in solution**

DABS-hydrazine is oxidized by $Cu^{2+}$ in aqueous solutions, and therefore the low recoveries of glucose in the Cu-clay systems (2.5 to 22.7%) are believed to be an artifact. DABS-hydrazine is also oxidize by $Fe^{3+}$ in aqueous solutions, however the high recoveries of glucose in the Fe-coated clays (78.2 to 91.9%) suggest that oxidation of DABS-hydrazine was not a problem, probably because little or no $Fe^{3+}$ was in the solutions. Thus, the results of the Cu-clay + glucose systems were not used in the data analysis but the results for the Fe-coated clays + glucose were used. Glucose recoveries in the clay mineral + glucose systems ranged from 18.3 to 98.3% of the total organic C added. Glucose recovery depended on the type of clay; Na-SWa > -Panther > -Otay = -Saponite; Ca-SWa > -Panther > -Otay > -Saponite and Al-SWa

> -Panther > -Saponite > -Otay; however, no significant differences were observed in glucose recoveries for the Fe-coated clays (Table 3b). Also, glucose recovery depended on the saturating cations; for SWa clay Na > Al = Fe > Ca; for Panther clay Fe > Na > Ca = Al; for Saponite clay Fe > Na = Ca = Al; and for Otay clay Fe > Ca > Na > Al (Table 3a). Glucose recoveries in the Al-substituted goethite + glucose systems were 58.3, 79.7, 80.1, and 83.2% for the Goe-00, -94, -20, and -51, respectively (Table 4).

## DISCUSSION

The smectites used in this study are classified as beidellite (Panther), montmorillonite (Otay), and saponite (Saponite). There is a debate whether SWa clay is a nontronite or not; for practical purposes, in this dissertation the SWa will be referred to as "ferruginous smectite" (Frost and Kloprogge, 2000). The main differences among the clay minerals are their chemical composition and the relative charge in the tetrahedral and octahedral sheets (Table 1). Montmorillonites are dioctahedral smectites characterized by relative low tetrahedral charge; beidellites are dioctahedral smectites characterized by relative high tetrahedral charge and saponites are Mg-rich trioctahedral smectites with relative high tetrahedral charge.

The *120, 130, 111*, and *140 hkl* reflections of goethite shift to lower *d*-values as Al substitution increases in goethite (Jonas and Solymar, 1970; Schulze, 1984) because the $Al^{3+}$ is smaller than the $Fe^{3+}$ (0.53 and 0.65 Å, respectively), which causes the unit cell of goethite to decrease as $Al^{3+}$ increases (Schulze, 1984). The Fe-O-H deformation band for goethites, about 900's $cm^{-1}$, which has been used to

estimate the degree of Al substitution in goethites (Jonas and Solymar, 1970; Schwertmann and Cornell, 2000), shifted from 892 to 911 $cm^{-1}$ as Al-substitution increased from 0 to 9.4% (mol/mol). No other significant changes in other FT-IR bands of the goethites used in this study were observed. The degree of Al-substitution for the goethites did not reach the level reported by Schwertmann and Cornell (2000) for similar preparations (0, 5.6, 13.5 and 27.3%, mol/mol Al/(Al+Fe)).The reason for the lower levels of Al-substitution in our goethites samples is not clear, however the degree of Al-substitution was different for each sample, which was our ultimate goal.

The complete recovery of soluble organic C together with the incomplete recovery of glucose, and the lack of evolved $CO_2$ suggests that glucose was abiotically transformed during the incubations with the smectites and Fe oxides. Soluble organic C was recovered almost completely; however, an overestimation was observed in some systems. The potassium dichromate oxidation method for measuring soil organic carbon (SOC) is based in the measurement of the unused potassium dichromate after oxidation of SOC. There are two conditions that overestimate the total organic C: (a) the presence of $Fe^{2+}$ and (b) the presence of $Cl^-$ according to the following reactions:

$$Cr_2O_7^{2-} + 6Fe^{2+} + 14H^+ = 2Cr^{3+} + 6Fe^{3+} + 7H_2O \qquad \text{(a)}$$

$$K_2Cr_2O_7 + 4KCl + 6H_2SO_4 = 2CrO_2Cl_2 + 6KHSO_4 + 3H_2O \qquad \text{(b)}$$

Most of the Fe in the system was in the ferric form ($Fe^{3+}$), however a small amount of ferrous ion ($Fe^{2+}$) was probably present as structural iron in the SWa and Panther clays and in the Al-substituted goethites and some $Fe^{2+}$ may have been present in the Fe-coated clays. The extent of the $Cl^-$ interference with the dichromate

oxidation method is small, and the same amount of $CaCl_2$ was added to all samples after incubations. Thus, the higher soluble and total organic C content in the SWa, Panther, Fe-coated clay, and goethite systems is believed to be due primarily to trace amounts of $Fe^{2+}$ in these systems.

There are three factors in the clay + glucose systems that could affect glucose recoveries: solution pH, type of clay and saturating cation. Solution pH of the systems ranged from 3.0 to 6.3. Both forms of glucose (cyclic and open chain structures) react most rapidly with hydrazine to form hydrazones in the pH 4 to 5 range. Thus, if solution pH influenced glucose recovery; higher recoveries would be expected either under acidic (pH < 4) or slightly acidic (pH > 5) conditions. In this study, however, both low and high glucose recoveries were obtained under acidic conditions (Al-Otay, pH 3.3, 18.3% and Al-SWa, pH 3.0 and 83.9% glucose recovery). And, both low and high glucose recoveries were also obtained under slightly acidic conditions (Ca-Saponite, pH 5.8, 43.9% and Fe-Saponite, pH 5.8, 83.9% glucose recovery). Thus, clay-type and saturating cation effects are clearly dominant over pH effects on glucose recoveries in this study.

Interestingly, $Fe^{3+}$ content has a positive effect on glucose recoveries. Glucose recoveries increased as structural $Fe^{3+}$ increased in Na-, Ca-, and Al-saturated clays (Table 3b), and high recoveries of glucose were obtained for all of the Fe-coated clays. Furthermore, relatively high glucose recoveries were obtained for the various goethite samples (Table 4). These results suggest that $Fe^{3+}$, probably Fe-oxyhydroxide surface coatings, inhibit the abiotic clay catalyzed transformation of glucose. However, significantly higher recoveries of glucose were obtained for the Al-substituted

goethites than the non-substituted goethite (Table 4). This could be a pH effect, or an effect of the Al-substitution on the nature of surface active sites of goethite.

Controls consisting of fresh 100 mM glucose in 100 mM $Fe(NO_3)_3$, and $Al(NO_3)_3$ solutions (1:1 v/v) were analyzed. There was no peak for glucose in the chromatogram for the glucose + $Fe(NO_3)_3$ system, indicating that $Fe^{3+}$ had oxidized all of the DABS-hydrazine. By contrast, there was nearly complete recovery of glucose from the glucose + $Al(NO_3)_3$ control. These results together with the fact that recoveries of glucose increased with Fe content and decreased for the Al-clays suggest that glucose is catalytically transformed by smectites into unknown compound (s) under conditions similar to those found in soils.

An attempt was made to identify the unknown transformation products of glucose. Based on the knowledge that glucose is dehydrated to furfural compounds and that furfural compounds are further transformed to levulinic acid and formic acid under highly acidic conditions, we hypothesized that clay surfaces may be catalyzing the hydration of glucose in our systems. An HPLC analysis of the solutions from several of the clay + glucose systems reveled small to negligible peaks for HMF and relatively large peaks for levulinic acid. Generally the peak intensity for the levulinic acid increased as glucose recovery decreased.

Shown in Fig. 5 are the chromatograms for furfural and levulinic acid analysis of the solution from the Al-Otay + glucose systems and aqueous controls (64 h at 70°C) containing glucose-only and a mixture of glucose and 1 M HCl. The levulinic acid peak is the dominant peak in this chromatogram for the glucose + HCl aqueous control but completely absent from the aqueous glucose-only control. Levulinic acid is

a known acid breakdown product of HMF (Ferrer et al., 2000) and was an anticipated product in the glucose + HCl control. Furthermore, the effects of metal salts on glucose dehydration were investigated. HMF and levulinic acid peaks were only observed for three metal salts: HMF ($CuCl_2$ > $Al(NO_3)_3$ > $Fe(NO_3)_3$) and levulinic acid ($Fe(NO_3)_3$ > $Al(NO_3)_3$ > $CuCl_2$). The $Al^{3+}$, $Fe^{3+}$ and $Cu^{2+}$ ions are stronger Lewis acids than $Na^{+}$ and $Ca^{2+}$ ions, thus, the acid-catalyzed dehydration is facilitated by those metals in aqueous systems. However, $Fe^{3+}$ is found as a structural ion or in surface coatings in the clay and goethite samples, whereas $Al^{3+}$ is free in solution; hence, glucose dehydration may be "inhibited" in Fe-rich minerals. Additionally, the cation-carbohydrate complex strength is affected by the ionic radii of cations: the best radius for complex formation is 100-110 pm ($Na^{+}$, $Ca^{2+}$) whereas smaller cations ($Fe^{3+}$ and $Al^{+3}$) form weaker complexes with carbohydrates (Angyal, 1989); thus, $Na^{+}$ and $Ca^{2+}$ may protect glucose from dehydration.

In hindsight, the furfural compounds are probably too unstable to have persisted in the solutions for the clay + glucose systems due to the acid-catalyzed cleavage of furfural compounds to form levulinic acid and formic acid. The results of this study demonstrate that clay minerals are capable of catalyzing the dehydration of glucose and the cleavage of HMF to yield of levulinic acid under conditions relevant to soil environments.

Carbohydrates are by far the most abundant form of biomass introduced into soils each year. As soil microorganisms degrade carbohydrates, some monosaccharides will be released to the soil solution. Although most of the monosaccharides will be consumed by microbes, some will reach the surfaces of soil

clays. The results of this study provide evidences that monosaccharides are abiotically transformed by smectites into HMF and ultimately into levulinic acid. The furfural compounds and their degradation products are known to be highly reactive, and thus are likely to be polymerized or co-polymerized with other organic compounds. Accordingly, the catalytic transformation of glucose by smectites is suggested as one of the pathways for the incorporation of new C into the SOM pool during the formation of new humic-like substances.

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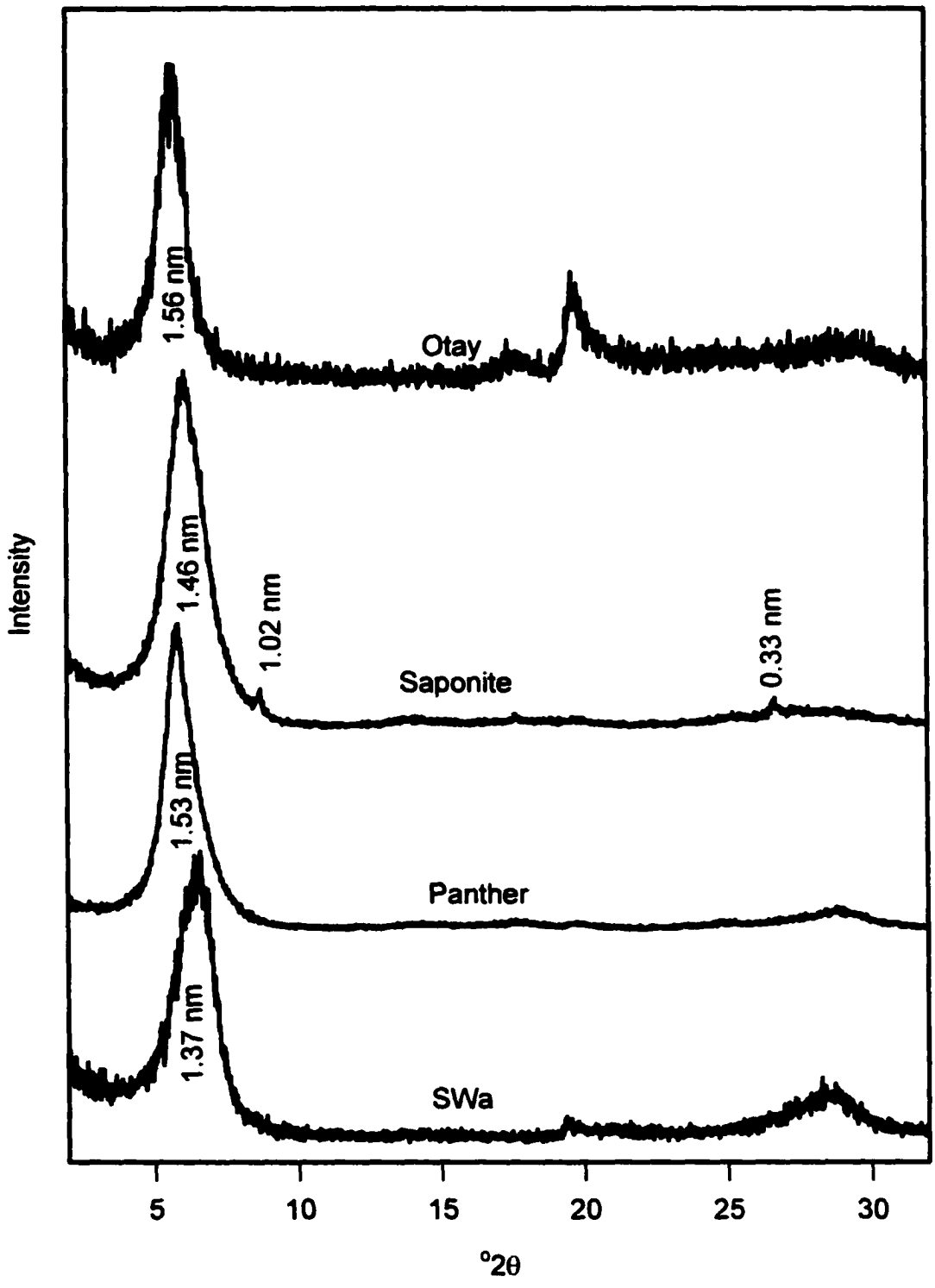


Fig. 1. X-ray diffraction patterns of Ca-saturated clays equilibrated at 54% relative humidity.

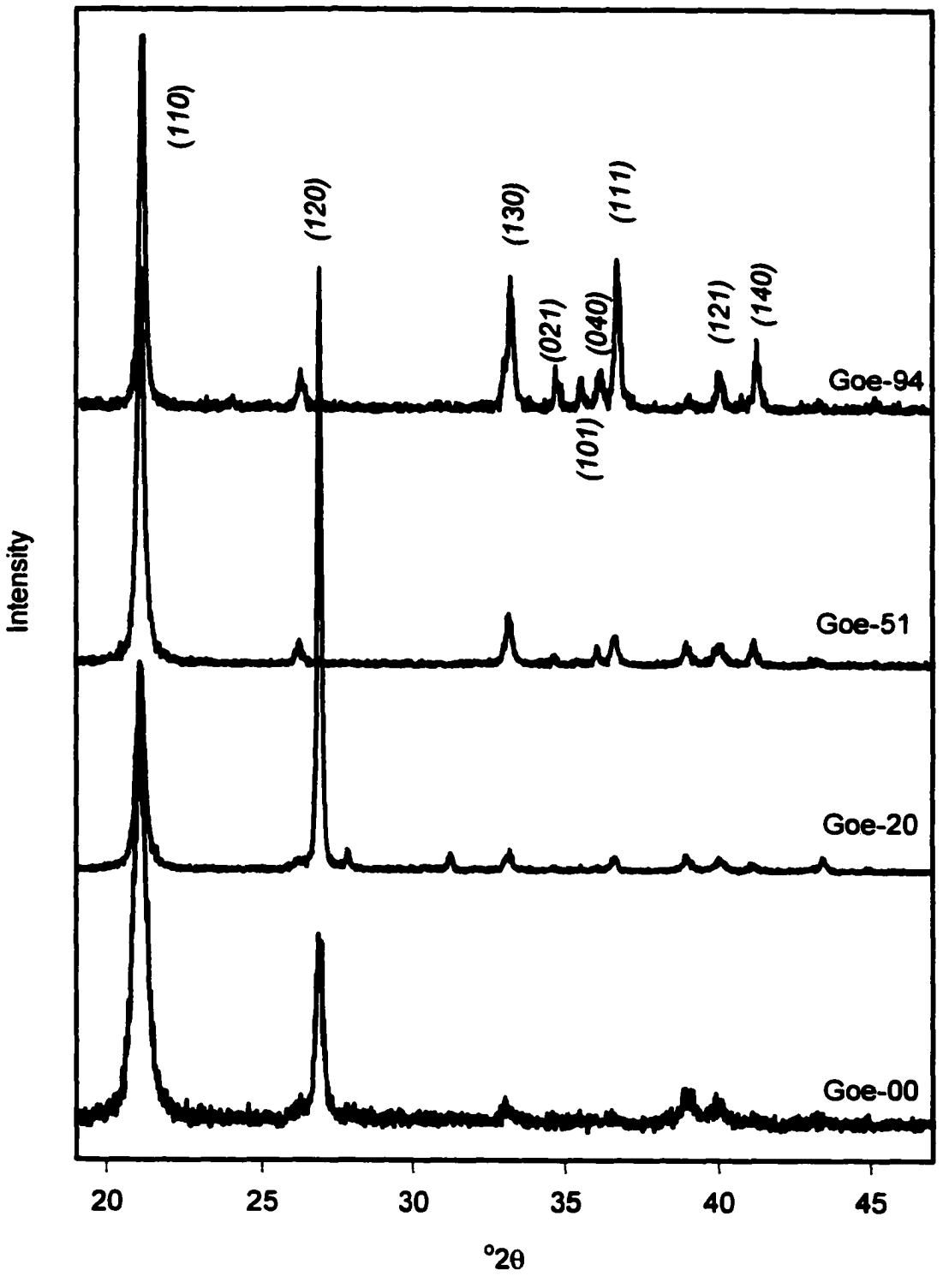


Fig. 2. X-ray diffraction patterns of Al-substituted goethites. Miller indices (*hkl*) in parenthesis.

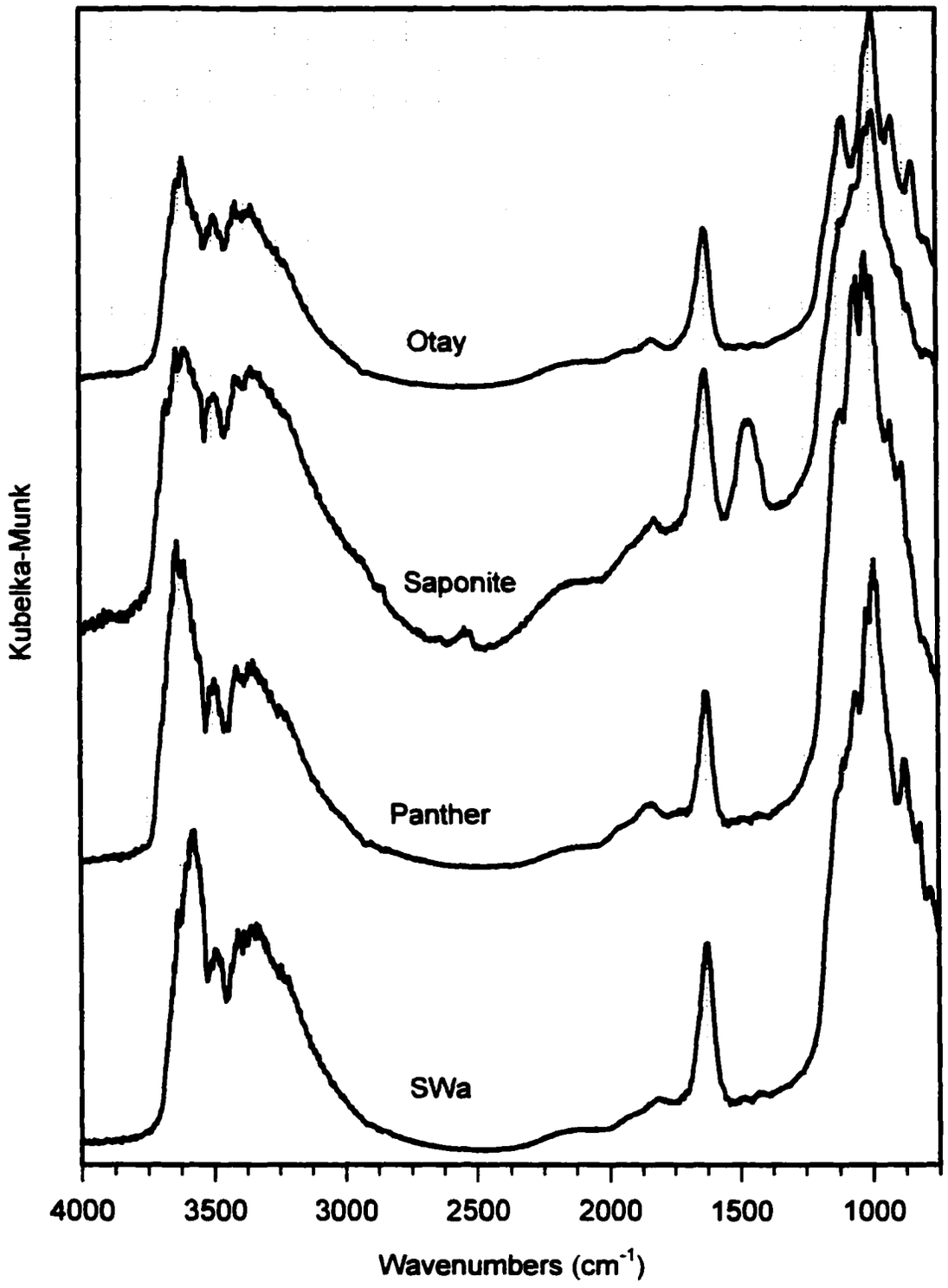


Fig. 3. FT-IR spectra of the Ca-Clays used in this study.

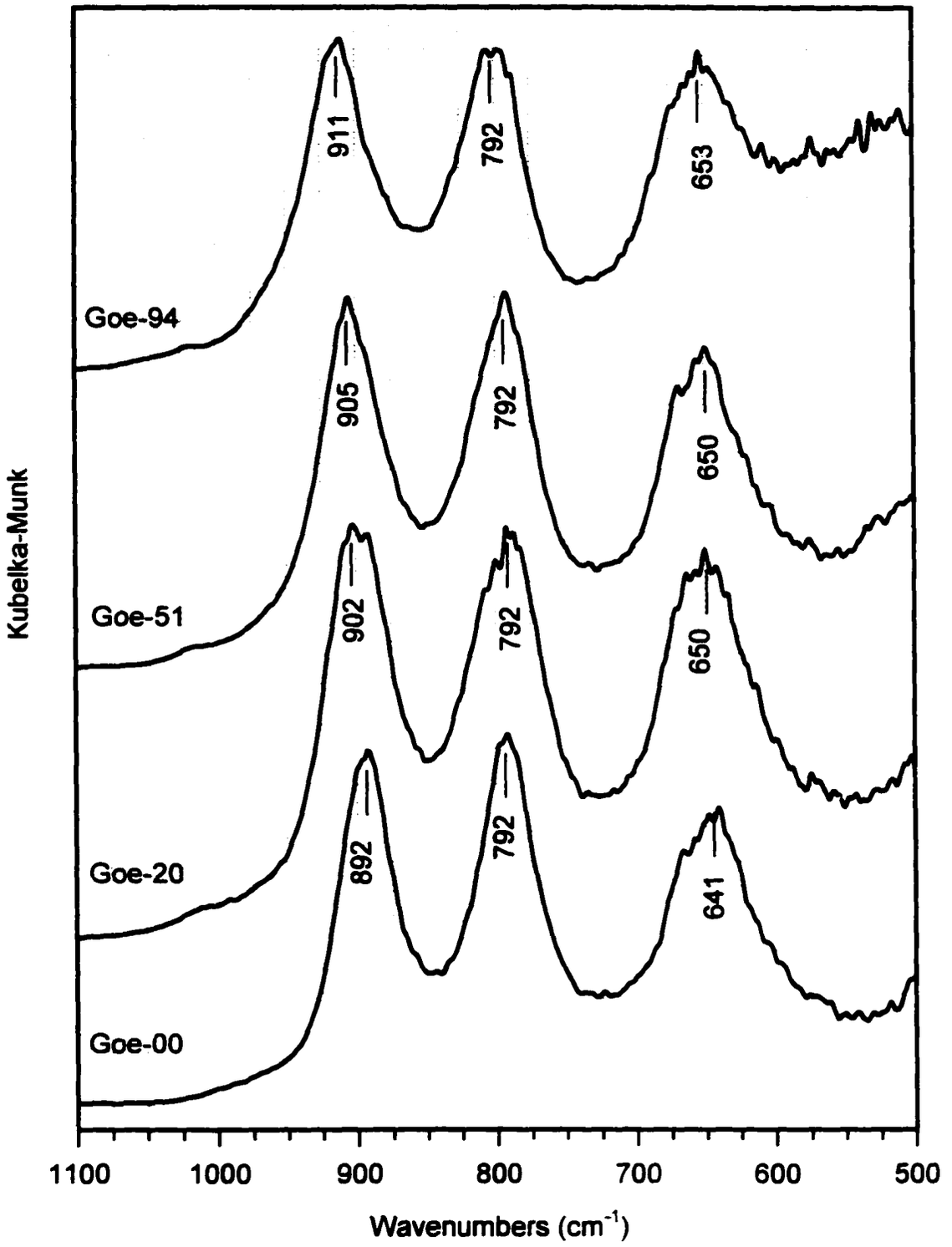


Fig. 4. FT-IR spectra of the Al-substituted goethites.

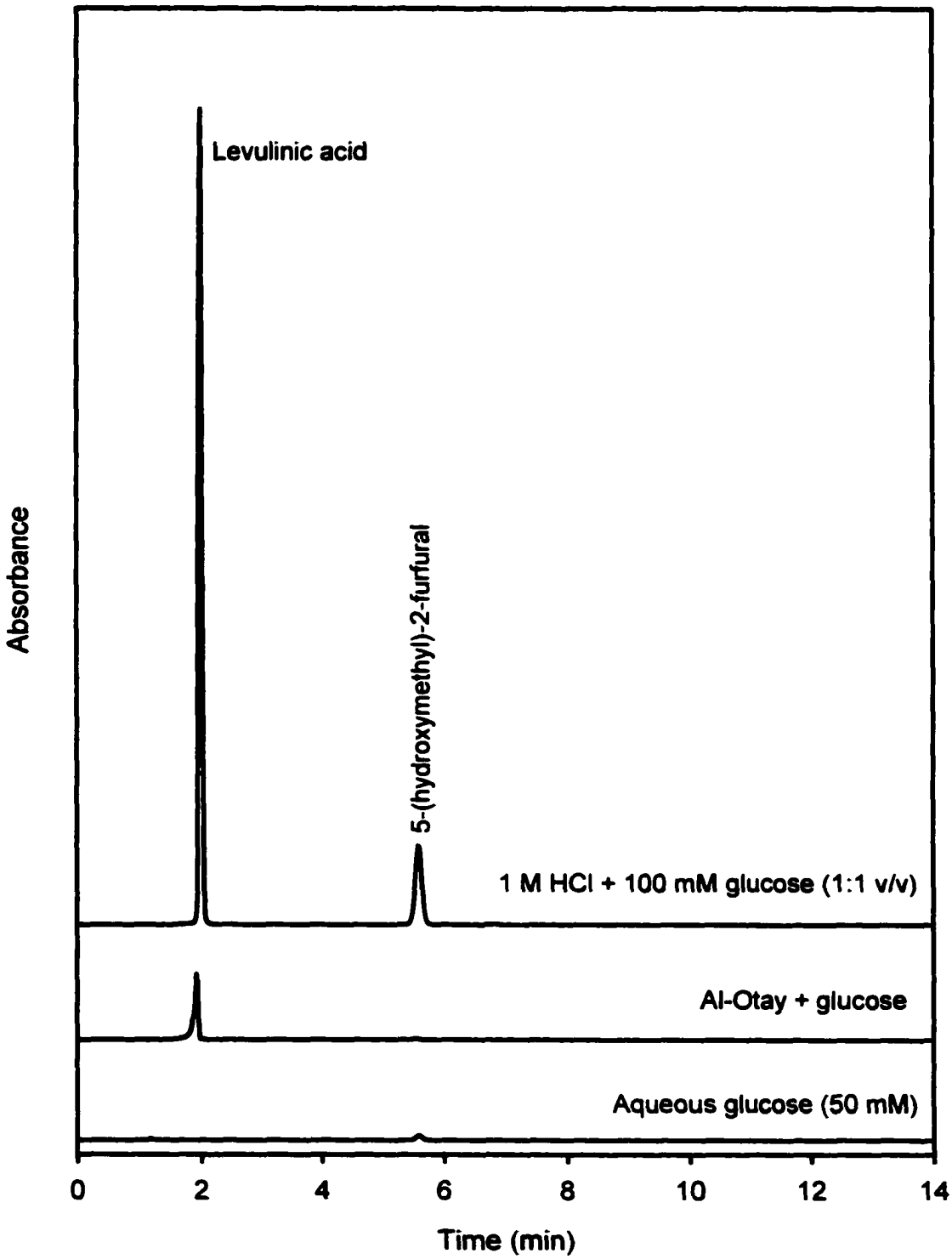


Fig 5. Chromatograms for furfural compounds of HCl-glucose and aqueous glucose (incubated 64 h at 70°C) and Al-Otay + glucose (incubated for 21 days at 37°C). Intensity is at the same scale for all chromatograms.

Table 1. Elemental analysis of the clay minerals used in this study.

| Clay Mineral | C | N | Interlayer Charge | Th‡ | | Oh§ | | | Th Charge | $Fe^{3+}$ coating clays¶ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $Si^{4+}$ | $Al^{3+}$ | $Al^{3+}$ | $Fe^{3+}$ | $Mg^{2+}$ | | |
| | -----% ----- | | | ---------- *cations per formula unit*# ---------- | | | | | --- % --- | -- *mg* -- |
| SWa | 0.21 | 0.02 | 0.46 | 3.70 | 0.30 | 0.41 | 1.44 | 0.14 | 64.0 | 145 |
| Panther | 0.11 | 0.01 | 0.38 | 3.86 | 0.14 | 1.43 | 0.35 | 0.21 | 36.7 | 124 |
| Saponite | 0.39 | 0.01 | 0.58 | 3.77 | 0.23 | 0.88 | 0.09 | 1.37 | 38.6 | 221 |
| Otay | nd† | nd | 0.53 | 3.99 | 0.01 | 1.32 | 0.06 | 0.67 | 2.23 | 169 |

† nd: not detected
‡ Th: Tetrahedral sheet
§ Oh: Octahedral sheet
¶ mg $Fe^{3+}$ coating 1 g clay
# Formula unit based in $M^{n+}_{x+y}(Si_{4-x}\ Al_x)(Al_{2-y-z}\ Mg_y\ Fe^{3+}_z)O_{10}(OH)_2$

Table 2. Al substitution in synthetic goethites used in this study.

| Sample | C | N | Al→Fe substitution‡ |
|---|---|---|---|
| | ---------- % ---------- | | % mol/mol |
| Goe-00 | 0.12 | nd† | 0.0 |
| Goe-20 | 0.12 | nd | 2.0 |
| Goe-51 | 0.15 | nd | 5.1 |
| Goe-94 | 0.10 | nd | 9.4 |

† nd: not detected

‡ $\%\ Fe\ substituted = \left(\frac{Al}{Al + Fe}\right) x 100$

Table 3a. Mass balance of C for the clay + glucose systems.

| Sample | pH | C in Solution | | $C\text{-}CO_2$ | C-Total‡ |
|---|---|---|---|---|---|
| | | Total† | C-Glucose# | | |
| | | % C Recovered | | | |
| ***SWa*** | | | | | |
| Na | 4.7 | 104.0 b§ | 98.3 c | 0.1 | 104.1 |
| Ca- | 5.2 | 102.7 b | 65.3 a | 0.1 | 102.8 |
| Cu- | 3.5 | 95.3 a | 22.7 ¶ | 0.0 | 95.3 |
| Al- | 3.0 | 109.6 c | 83.9 b | 0.0 | 109.6 |
| Fe- | 3.3 | 108.2 c | 82.0 b | 0.1 | 108.2 |
| ***Panther*** | | | | | |
| Na | 3.8 | 101.4 b | 81.2 b | 0.1 | 101.4 |
| Ca- | 3.9 | 102.0 b | 56.9 a | 0.0 | 102.0 |
| Cu- | 3.5 | 101.8 b | 2.5 | 0.0 | 101.8 |
| Al- | 3.2 | 95.0 a | 59.3 a | 0.0 | 95.0 |
| Fe- | 3.5 | 108.3 c | 91.9 c | 0.1 | 108.4 |
| ***Saponite*** | | | | | |
| Na | 6.2 | 101.3 b | 39.6 a | 0.1 | 101.4 |
| Ca- | 5.8 | 102.8 b | 43.9 a | 0.0 | 102.8 |
| Cu- | 4.2 | 95.5 ab | 10.4 | 0.0 | 95.5 |
| Al- | 3.9 | 99.0 b | 20.7 a | 0.0 | 99.0 |
| Fe- | 5.8 | 105.6 cb | 83.9 b | 0.2 | 105.8 |
| ***Otay*** | | | | | |
| Na | 6.3 | 102.9 ab | 43.9 b | 0.0 | 102.9 |
| Ca- | 5.9 | 105.3 b | 50.7 c | 0.0 | 105.3 |
| Cu- | 4.1 | 102.4 ab | 4.0 | 0.0 | 102.4 |
| Al- | 3.3 | 101.4 a | 18.3 a | 0.0 | 101.4 |
| Fe- | 3.7 | 109.3 c | 78.2 d | 0.0 | 109.3 |

† Determined by a modified dichromate oxidation method (Yeomans and Bremner, 1988).
‡ Sum of Total C in Solution and $C\text{-}CO_2$.
§: Within each clay mineral and within each C-fraction, means followed by the same letter are not significant different by the Student-Newman-Keuls test ($P<0.05$).
¶ C-glucose for Cu-saturated clays was not used for the statistical analysis.
# Glucose recovery for control (aqueous solution of glucose) was 88.0%.

Table 3b. Mass balance of C for the clay + glucose systems.

| Sample | pH | C in Solution | | $C\text{-}CO_2$ | C-Total‡ |
|---|---|---|---|---|---|
| | | Total† | C-Glucose# | | |
| | | % C Recovered | | | |
| ***Na*** | | | | | |
| SWa | 4.7 | 104.0 a§ | 98.3 c | 0.1 | 104.1 |
| Panther | 3.8 | 101.4 a | 81.2 b | 0.1 | 104.1 |
| Saponite | 6.2 | 101.3 a | 39.6 a | 0.1 | 101.4 |
| Otay | 6.3 | 102.9 a | 43.9 a | 0.0 | 102.9 |
| ***Ca*** | | | | | |
| SWa | 5.2 | 102.7 a | 65.3 d | 0.1 | 102.8 |
| Panther | 3.9 | 102.0 a | 56.9 c | 0.0 | 102.0 |
| Saponite | 5.8 | 102.8 a | 43.9 a | 0.0 | 102.8 |
| Otay | 5.9 | 105.3 b | 50.7 b | 0.0 | 105.8 |
| ***Cu*** | | | | | |
| SWa | 3.5 | 95.3 a | 22.7 ¶ | 0.0 | 95.3 |
| Panther | 3.5 | 101.8 b | 2.5 | 0.0 | 101.8 |
| Saponite | 4.2 | 95.5 a | 10.4 | 0.0 | 95.5 |
| Otay | 4.1 | 102.4 b | 4.0 | 0.0 | 102.4 |
| ***Al*** | | | | | |
| SWa | 3.0 | 109.6 c | 83.9 d | 0.0 | 109.6 |
| Panther | 3.2 | 95.0 a | 59.3 c | 0.0 | 95.0 |
| Saponite | 3.9 | 99.0 b | 20.7 b | 0.0 | 99.0 |
| Otay | 3.3 | 101.4 b | 18.3 a | 0.0 | 101.4 |
| ***Fe*** | | | | | |
| SWa | 3.3 | 108.2 a | 82.0 a | 0.1 | 108.2 |
| Panther | 3.5 | 108.3 a | 91.9 a | 0.1 | 108.4 |
| Saponite | 5.8 | 105.6 a | 83.9 a | 0.2 | 105.8 |
| Otay | 3.7 | 109.3 a | 78.2 a | 0.0 | 109.3 |

† Determined by a modified dichromate oxidation method (Yeomans and Bremner, 1988).
‡ Sum of Total C in Solution and $C\text{-}CO_2$.
§: Within each clay mineral and within each C-fraction, means followed by the same letter are not significant different by the Student-Newman-Keuls test ($P<0.05$).
¶ C-glucose for Cu-saturated clays was not used for the statistical analysis.
# Glucose recovery for control (aqueous solution of glucose) was 88.0%.

Table 4. Mass balance of C for the goethite + glucose systems.

| Sample | pH | C in Solution | | $CO_2$-C | Total C‡ |
|---|---|---|---|---|---|
| | | Total† | Glucose-C | | |
| | | % C Recovered | | | |
| Goe-00 | 4.7 | 108.2 a§ | 58.3 a | 0.0 | 108.2 |
| Goe-20 | 3.9 | 106.0 a | 80.1 b | 0.0 | 106.0 |
| Goe-51 | 3.9 | 106.1 a | 83.2 b | 0.0 | 106.1 |
| Goe-94 | 3.8 | 110.1 a | 79.7 b | 0.0 | 110.1 |

† Determined by a modified dichromate oxidation method (Yeomans and Bremner, 1988).
‡ Sum of Total C in Solution and C-$CO_2$.
§ Within C-fraction, means followed by the same letter are not significant different by the Student-Newman-Keuls test ($P<0.05$).
¶ Al/(Al+Fe) molar ratio

# CHAPTER 4

# ROLE OF SMECTITES ON THE CATALYTIC POLYMERIZATION OF ARGININE AND GLUCOSE

A paper to be submitted to Clays and Clay Minerals

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## ABSTRACT

The mechanisms of soil organic matter formation have been studied for a long time. The polyphenol theory of humic substance formation has been extensively studied; however, an alternative theory that humic substances are formed through the condensation of amino acids and reducing sugars (Maillard reaction) has not been extensively explored. The general objectives of this study were to determine whether smectites and goethites catalyze the abiotic polymerization of arginine and glucose to form humic-like compounds. The effects of smectite type, saturating cation, and the degree of Al-substitution in goethites on the polymerization reaction were also studied. Four cation-saturated smectites and four Al-substituted goethites were abiotically incubated with solutions containing a mixture of arginine + glucose for 21 days at 37°C. After the incubations, total C recovered ranged from 80.6 to 123.8% and from 100.5 to 105.1% for the clay and goethites systems, respectively. However, the

difference between total soluble organic C and glucose-C + arginine-C ranged from 7.1 to 33.7% and from 19.7 to 29.2% for clay and goethite systems, respectively. Thus, it is suggested that arginine and glucose were abiotically transformed by smectites. The Cu-clays strongly sorbed arginine (from 84.0 to 90.0% arginine-N), whereas Al-substituted goethites showed little sorption of arginine (from 2.3 to 4.6% arginine-N). The XRD data show that arginine is intercalated in the interlayer spaces of smectites.

## INTRODUCTION

Soil organic matter (SOM) has important roles in soils. SOM enhances the stabilization of the soil structure; consequently aeration, water-holding capacity and permeability of soils are also improved. Furthermore, SOM increases nutrient availability to plants via mineralization of organic forms of N, P, S and other nutrients. From an environmental point of view, organic pollutants and heavy metals are prevented from contaminating water by binding to SOM. Additionally, C sequestration in SOM has been suggested as one of the means of mitigating the increase of greenhouse gases in the atmosphere.

SOM can be fractionated into humic and nonhumic substances. Humic substances are high-molecular-weight substances formed from secondary synthesis reactions. Nonhumic substances include known classes of biochemical compounds such as amino acids, carbohydrates, waxes, etc. There are four major theories for the formation of humic substances. The oldest theory proposed that lignin is partially used by organisms, and the modified lignin residue becomes part of the SOM (Stevenson,

1982; Stevenson and Cole, 1999). The second theory hypothesized that the by-products (phenolic compounds and organic acids) of lignin decomposition are condensed and polymerized to form humic substances (Stevenson, 1982; Stevenson and Cole, 1999). The third theory also hypothesized that polyphenols are the building blocks of humic substances; however, the starting materials are hypothesized to be microbial transformation products from non-lignin C sources. These polyphenols are oxidized to quinones and ultimately polymerized to form humic substances (Stevenson, 1982; Stevenson and Cole, 1999). The fourth theory suggests that Maillard reactions products are the building blocks for SOM (Stevenson, 1982; Stevenson and Cole, 1999).

The polyphenol theory for formation of humic materials has been widely studied. Both biotic and abiotic catalyzed polymerization of starting materials has been studied. Microorganisms are capable of transforming small organic molecules into humic-like substances (Haider and Martin, 1970; Martin et al., 1972; Pal et al., 1994; Saiz-Jimenez et al., 1975; Sulfita and Bollag, 1981). It has been reported that enzyme-catalyzed reactions (laccase, a *p*-diphenol oxidase from fungi) were more effective for oxidizing phenols than abiotic-catalyzed reactions with birnessite, a Mn oxide (Pal et al., 1994).

Clay minerals (smectites, illite and kaolinite) and various Fe, Al and Mn oxides have been reported to serve as catalysts for the formation of humic-like substances from mixtures of phenols and glycine (Lehmanh et al., 1987; Naidja et al., 1998; Wang, 1991; Wang and Huang, 1989; Wang and Huang, 1991; Wang, 1993; Wang, 1987; Wang et al., 1978; Wang et al., 1983). The catalytic power of clay minerals to form

humic substances decreases in the order: smectite > illite > kaolinite > quartz (Wang et al., 1978).

The non-enzymatic condensation of melanoidins (insoluble brown nitrogenous containing compounds) from mixtures of amino acids and reducing sugars is known as the Maillard reaction. Maillard reactions have been investigated extensively from the geological and food sciences point of view. After a 5-day incubation of glucose + glycine at 60°C, 25% of the glucose was consumed to form melanoidins (Reyes et al., 1982). Furthermore, it has been reported that basic amino acids are more reactive towards reducing sugars than neutral or acid amino acids to form melanoidins (Hayase et al., 1996; Hedges, 1978; Yamamoto and Ishiwatari, 1989). Clay minerals (smectites and illites) have been shown to catalyzed the Maillard reaction when samples are subjected to "wetting-drying" cycles at relative high temperatures (70 to 100 °C) mimicking pre-biotic conditions on the earth (Arfaioli et al., 1997; Arfaioli et al., 1999; Bosetto et al., 2002; Bosetto et al., 1994). Although there is vast amount of information on the role of clay minerals in catalyzing the condensation of melanoidins from the geological point of view, there is little information on the ability of smectites to abiotically catalyze the condensation of amino acids and reducing sugars under conditions found in soil environments.

Stevenson (1982) and Stevenson and Cole (1998) reported that from 65 to 80% of the total N in soils is acid hydrolyzable N. Furthermore, it has been reported that amino acids accounted for 32 to 50% of the organic N in soils collected from two long-term cropping studies (Senwo and Tabatabai, 1998). Using a size fractionation approach, Laird and co-workers (2001) found that 76 to 97% and 4 to 6% of the

extracted N from the clay-associated organic matter was accounted for amino acids and amino sugars, respectively. Arginine, a basic amino acid, was the most abundant amino acid (50 to 66% of the total N) in the clay fractions of the soils studied by these researchers. It was reported that only 30 to 52% of the total C in the different size-clay fractions was extracted as monosaccharides, amino sugars, amino acids, and fatty acids (Laird et al., 2001).

Smectites are considered both Lewis and Bronsted acids. Exposed Si and tetrahedrally or octahedrally coordinated Al and Fe, when hydrated, are weak Bronsted acids (Heller-Kallai, 2002). The acidic character of smectites increases with the polarizing power of the exchangeable cations and decreasing water content (Heller-Kallai, 2002). Smectites, because of their surface acidity, can catalyze some organic reactions including: Diels-Alder cycloadditions (Heller-Kallai, 2002; Laszlo, 1987), Friedel-Crafts reactions (Heller-Kallai, 2002; Laszlo, 1987), aldol reactions(Izumi, 1992), Michael additions reactions (Izumi, 1992), and oxidation reactions (Heller-Kallai, 2002). Thus, it is hypothesized that smectites catalyze the condensation of amino acids and sugars under conditions similar to those found in soil environments. On the other hand, there is lack of information of the role of Fe oxides on the catalytic polymerization of amino acids.

The specific objective of this study were: (1) to determine whether smectites (type of smectite) and their associated saturating metal cations catalyze the abiotic co-polymerization of arginine and glucose to form humic-like compounds and (2) to determine if synthetic Al-substituted goethites can catalyze the abiotic co-polymerization of arginine and glucose to form humic-like compounds.

# MATERIALS AND METHODS

## Materials

Chemicals: L-arginine (>98% purity) and ACS reagent grade D-glucose (Sigma-Aldrich Corp., St. Louis, MO) were used without further purification. DABS-Cl [4-(Dimethylamino)azobenzene-4-sulfonyl chloride] (96% purity) (Sigma-Aldrich Group, St. Louis, MO) was used for the pre-column derivatization of amino acids. Milli-Q water (Milli-Q system, Millipore, Bedford, MA) with a resistivity of 18.2 MΩ-cm was used to prepare all solutions.

## Samples preparation

Sample preparation has been described in Chapter 3 of this dissertation. Briefly, four reference smectites, Panther Creek beidellite (Panther), Otay white montmorillonite (Otay), IMV saponite (Saponite), and ferruginous smectite (SWa) were separated (the clay fraction <2 μm) from the bulk ore by sedimentation. The clays were Na-, Ca-, Cu-, and Al-saturated by washing the clays twice with a 1 M solution and twice with a 0.1 M solution of the respective metal salts. A portion of the Na-saturated clays was coated with $Fe(OH)_3$ polymers made from a dialyzed $Fe(NO_3)_3$ solution (Rengasamy and Oades, 1977). The excess salt was removed by dialyzing the cation-saturated clays against Milli-Q water until the conductivity of dialysate was <0.6 mS/m. The cation-saturated clay samples were freeze-dried and saved for later use.

Four Al-substituted goethites with different Al/(Fe+Al) mol/mol ratios were synthesized (Schwertmann and Cornell, 2000). Briefly, 0, 20, 50, and 120 mL of

0.3125 M aluminate solution and 180, 178, 174, and 165 mL, respectively, of 5.0 M KOH were mixed in polyethylene containers. Then, 100 mL of freshly prepared 1.0 M $Fe(NO_3)_3$ solution were added quickly to each container, the samples were diluted to 2 L with distilled water, mixed and placed in the oven for 14 days at 70°C. After crystallization, the samples were centrifuged to separated the precipitate, and the precipitate was washed twice with 400 mL 1.0 M KOH to remove excess Al, then the pH was adjusted to 7.5 with HCl, washed once with distilled water, and freeze dried.

**Incubation**

About 0.25 or 0.50 g of $M^{n+}$-saturated clays or Al-substituted goethites were placed in 15-mL amber vials with open-top phenolic screw caps and loosely capped using PTFE/silicone septa (Supelco, Bellefonte, PA). The capped vials were sterilized in an autoclave for 15 min at 120 °C. The arginine and glucose solutions were prepared fresh for each experiment, filter-sterilized with 150-mL Nalgene® MF75 Series sterile disposable tissue culture filter units (Nalge Nunc International, Rochester, NY), and added to the cooled-vials containing the sterilized clay or Fe oxide under sterile conditions to avoid microbial contamination. The final concentration of arginine and glucose was about 0.5 mmol $g^{-1}$-clay or Fe oxide each, or a combined total of 1.0 mmol $g^{-1}$ clay or Fe oxide. The total solution volume was 5 mL. The solutions were dispended using a Repeater® Plus pipet with autoclave-sterilized 50-mL Eppendorf Combitip Plus tips (Eppendorf AG, Hamburg, Germany). The vials containing the $M^{n+}$-saturated clay or Al-substituted goethite + arginine + glucose systems were capped tightly and a strip of wax paper was wrapped

around the caps to further insure the integrity of the systems. The vials were vortex-mixed and incubated in the dark for 21 days in a temperature-controlled incubator at 37±0.5 °C. Three replicates were run for each treatment.

**Analysis**

After incubation, the evolved carbon dioxide concentration was measured with a $CO_2$ infrared gas analyzer (for details, refer to Chapter 3 of this dissertation). After the $CO_2$ analysis, 5-mL of 100 mM filter-sterilized $CaCl_2$ solution were added to the vials under sterile conditions, mixed and centrifuged for 10 min at 4500 x *g*. The supernatant was analyzed for arginine, glucose and total organic C and the pH of the supernatant was measured.

Arginine was analyzed by reverse phase-HPLC using pre-column derivatization of arginine with 4 mM DABS-Cl solution. The DABS-Cl was dissolved in acetonitrile, filtered with No. 40 Whatman filter paper and stored in amber bottles at 4°C. The pre-column derivatization or "dabsylation" procedure was as follows: A 25-μL sample aliquot, 500 μL of 50 mM $NaHCO_3$ buffer solution (pH 9.0) and 750 μL of 4 mM DABS-Cl solution were added to a clear 2-mL screw cap sample vial with Bakelite/PTFE liner cap (Supelco, Bellefonte, PA), capped and reacted for 30 min in a temperature-controlled block heater (Supelco, Bellefonte, PA) set at 70 °C. The analysis was performed by using a HP 1050 quaternary pump and an 1100 diode array detector (Hewlett-Packard, Wilmington, DE). A reverse phase ODS $C_{18}$ column (4.6 by 150 mm, 5 μm particle size) (Supelco, Bellefonte, PA) was used. The injection was performed using a 10-μL loop and a Rheodyne 7125 syringe loading injector

(Rohnert Park, CA). Absorbance was measured at 470 nm. The mobile phase consisted of acetonitrile (A) and a mixture of 40 mM sodium acetate (pH 6.5)/acetonitrile (v/v 85:15) (B). A flow rate of 1 mL $min^{-1}$ was used with the following gradient profile: 0 min 25% A, 75% B; 3.0 min 25% A, 75% B; 7.0 min 55% A, 45% B; 11 min 95% A, 55 B; 15 min 95% A, 5% B; re-equilibration time with 25% A, 75% B was 3 min. The retention time for arginine was 4.9 min. Calibration was performed using external standards. At least two other peaks were observed in all chromatograms, excess DABS-Cl and $NH_4^+$ (2.6 and 9.4 min, respectively). The $NH_4^+$, a degradation product of arginine, was quantified by dabsylation using $NH_4Cl$ external standards.

The procedure for glucose analysis has been described in Chapter 3 of this dissertation. Briefly, a 25-µL aliquot of supernatant was placed in a 2-mL clear vial and dried under a stream of $N_2$ gas in a sand bath at 40 °C. To the dried sample, 400-µL of DABS-hydrazine solution was added, capped and reacted for 30 min at 70 °C. The solution was allowed to cool at room temperature and immediately analyzed by reverse phase-HPLC. The mobile phases consisted of acetonitrile (A) and a mixture (v/v 40:60) of acetonitrile/water (pH 3.0 with HPLC-grade $H_3PO_3$) (B). A flow rate of 0.8 mL $min^{-1}$ was used with the following gradient profile: 0 min 100% B; 6.0 min 100% B; 10 min 10% B; 15 min 10% B, with re-equilibration of 5 min. The retention time of glucose was 5.6 min. Calibration was performed using external standards. Peaks for the un-reacted DABS-Cl and DABS-hydrazine were observed at 3.0 and 14 min, respectively.

Organic carbon analysis in the supernatant was described in Chapter 3 of this

dissertation. Briefly, an aliquot of 2.0 mL of supernatant, 5.0 mL of 1 N $K_2Cr_2O_7$ and 7.5 mL of concentrated $H_2SO_4$ were boiled for 15 min in an Erlenmeyer flask, connected to a condenser. The flasks were allowed to cool at room temperature, 0.3 mL of indicator solution was added, and the samples were titrated with Mohr's salt solution. Two boiled controls and two unboiled controls were also prepared.

After the analysis of arginine, glucose, total organic C, and pH were complete, the supernatant was decanted. The sediment was rinsed with water and centrifuged for 10 min at 4000 x *g* several times until the conductivity of the supernatant was <0.6 mS/m to remove excess salt and organic compounds. Then, the samples were freeze-dried. The total C and total N in the freeze-dried samples were determined by dry combustion using a Carlo-Erba NA1500 NSC elemental analyzer (Haake Buchler Instruments, Paterson, NJ). X-ray diffraction (XRD) patterns and infrared spectra for the freeze-dried treated and untreated samples were obtained (for details, see Chapter 3 of this dissertation). One Way Analysis of Variance (ANOVA) and pairwise multiple comparison procedures using the Student-Newman-Keuls Method ($P<0.05$) were performed with the SigmaStat software (SPSS Science, Chicago, IL).

## RESULTS

### Characterization of the Clays and Fe oxides Minerals

The four clay minerals and Fe oxides used in this study were described in Chapter 3 of this dissertation. In summary, typical XRD patterns for smectites were observed. In addition, the XRD patterns for saponite showed small illite and quartz

peaks. The four smectites are classified as "ferruginous smectite" (SWa), beidellite (Panther), montmorillonite (Otay) and saponite (Saponite). The main differences among the clay minerals are their composition and the relative charge in the tetrahedral and octahedral sheets. The total C and N content in the clays used in this study were from 0 to 0.39% and from 0 to 0.2%, respectively.

Al-substituted goethites were named Goe-00, Goe-20, Goe-51, and Goe-94 after their levels of Al-substitution (0, 2.0, 5.1, and 9.4% mol/mol Al /(Al+Fe), respectively). The total C content ranged from 0.10 to 0.15%, whereas total N content was negligible. All four samples showed typical XRD goethite reflections *(hkl)*:*110, 120, 130, 111, 140, 021, 101, 040* and *121*. FT-IR spectra for all four Al-substituted goethites show that the Fe-O-H deformation band for Goe-00, -20, -51, and -94 were 892, 902, 905, and 911 $cm^{-1}$, respectively.

**C mass balance**

The C mass balances for the clay systems within cations and within clays are presented in Tables 1a and 1b, respectively. Total C recovered ranged from 78.5 to 123.8%. The total C was variously distributed as soluble organic C (42.1 to 90.0%), arginine-C (0.9 to 22.5%), glucose-C (7.8 to 48.2%), sorbed C (14.9 to 50.3%) and $CO_2$-C (0 to 2.1%).

In the solution phase, glucose-C recovery (Table 1a) was not significantly different among cations for SWa and Panther clays, whereas the cation effect on glucose-C recovery was significant for the Saponite and Otay clays (Na > Ca = Al = Fe). The lowest arginine-C recovery from the solutions was found for the Cu-clay

systems (0.9 to 2.0%). Significant differences in arginine-C recovery were observed for SWa (Na = Ca = Al = Fe > Cu), Panther (Fe > Ca > Na > Al > Cu); Saponite (Fe > Na = Ca =Al > Cu), and Otay (Fe > Ca = Al > Na > Cu). Thus, higher arginine-C recoveries were observed for the Fe-coated clays, whereas lower arginine-C recoveries were found for the Cu-saturated clays. Soluble organic C recoveries show significant differences for SWa (Na = Al > Ca > Cu = Fe), Panther (Ca ≥ Cu = Al = Fe ≥ Na), Saponite (Fe ≥ Ca ≥Al > Na > Cu), and Otay (Fe ≥Ca ≥ Al Na = Cu). The highest sorbed C was found in the Cu-clays (42.5 to 47.3%); whereas the lower sorbed C was found for the Ca- and Na-clays (12.0 to 23.1%), except for the Na-Saponite (33.4%). Evolved $CO_2$-C was relative small for all clays; SWa (0 to 2.5%), Panther (0 to 1.9%), Saponite and Otay (0 to 0.3%).

Glucose-C recoveries from the solutions were not significantly different among clays for the Al-saturated and Fe-coated clay systems (Table 1b). However, glucose-C recoveries were significantly different for the Na- (SWa > Panther = Saponite = Otay), Ca- (Panther > Saponite = Otay > Panther), and Cu-saturated (Saponite > SWa = Panther = Otay) clay systems. Arginine-C recoveries from the solutions were not significantly different for the Na-, Ca-, and Fe-clay systems; however, significant differences among clays were observed for Al- (SWa = Saponite > Otay > Panther) and Cu-saturated (Panther > SWa = Saponite > Otay) clay systems. Among clays, soluble organic C was not significantly different for Ca-, Cu-, and Fe-clay systems; however, soluble organic C recoveries was significantly different for the Na- and Al-saturated clays (SWa > Saponite = Clay > Panther). The sum of the glucose-C and arginine-C was lower than the soluble organic C by 11.1 to 55.8%.

Glucose-C recoveries for the Cu-clays were not used for the statistical analysis because of hydrazine oxidation by $Cu^{2+}$ (see Chapter 3).

Carbon mass balances for the Al-substituted goethite + arginine + glucose systems are presented in Table 3. From 100.5 to 105.1% was recovered for the Al-substituted goethite + arginine + glucose systems. No microbial activity was assumed during incubation, evolved $CO_2$-C ranged from 0 to 0.9%. Among the Al-substituted goethites, no significant differences were observed for arginine-C (from 28.8 to 30.1), glucose-C (from 37.5 to 40.4), and sorbed C (from 6.2 to 9.0%); whereas, soluble organic C (from 91.4 to 98.3%) was significantly different among goethites (Goe-00 > Go0-51 = Goe-94 > Goe-20). The difference between soluble organic C and arginine-C + glucose-C in solution ranged from 19.6 to 29.5%.

**N mass balance**

The effects of saturating cation and clay type on N mass balances are presented in Tables 2a and 2b, respectively. From 55.8 to 93.4% of the total N was recovered (arginine-N + $NH_4^+$-N + sorbed N). Arginine-N ranged from 1.9 to 44.7%, $NH_4^+$-N from 0.3 to 16.5% and sorbed N from 16.5 to 90% of the total added. Arginine-N recoveries from the solutions showed significant differences among cations for SWa (Na = Ca = Al = Fe > Cu), Panther (Fe > Ca > Na > Al > Cu), Saponite (Fe > Na = Ca = Al > Cu), and Otay (Fe > Ca = Al > Na > Cu) (Table 2a). Sorbed N was significantly different among cations (Cu > Al > Fe) (Table 2a). In general, among cations $NH_4^+$-N content increase as sorbed N decreased (Table 2a). Among clays, arginine-N recoveries were not significantly different for the Na, Ca and Fe systems,

whereas significant differences were observed in the arginine-N recoveries for the Cu and Al systems (Table 2b). Overall, among clays, no significant differences were observed in the $NH_4^+$-N content (Table 2b). Sorbed N was relatively stable for the Ca- (26.4 to 35.7%), Cu- (84.0 to 90.0%) and Fe-coated (20.8 to 29.3%) clays. For the Al-saturated clays, however, sorbed N ranged from 38.8 to 63.5%. For Na-clays, sorbed N was determined only for the Saponite and Otay clays (16.5 and 40.6 %, respectively).

Mass balances for the Al-substituted goethite + arginine + glucose systems are presented in Table 4. Sorbed N was low, from 2.3 to 4.6%; arginine-N ranged from 58.2 to 59.9%; $NH_4^+$-N from 7.2 to 7.7%, and total N recovered from 67.5 to 71.1%. The only significant differences were for sorbed N (Goe-94 > Goe-00 > Goe-20 = Goe-51).

**XRD analysis of samples**

The XRD analysis was performed for the untreated and treated (arginine + glucose) samples equilibrated at 54% R.H. (atmosphere above a saturated $Mg(NO_3)_2$ saturated solution). The XRD patterns for the untreated Ca-saturated clays were presented in Chapter 3 of this dissertation. The *d*-spacings for all of the treated clays, except the Fe-coated clays, are restricted to a relatively narrow range (1.34 to 1.41 nm). The Fe-coated SWa had a *d*-spacing of 1.38 nm; however, *d*-spacings for the other Fe-coated clays are between 1.50 and 1.56 nm. By contrast, the untreated clays have a much widerange of *d*-spacing (1.22 to 1.59 nm).

**FT-IR**

FT-IR spectra for all $M^{n+}$-Otay + Arginine + Glucose systems, untreated Ca-Otay and arginine (crystalline form) are shown in Fig. 2. The untreated Ca-Otay showed a symmetrical absorption band about 1626 $cm^{-1}$ for the HOH deformation of water. The arginine spectrum is complex and at least eight peaks can be observed in the 1200 to 1800- $cm^{-1}$ range. Identified peaks in this region are; 1720 ($\nu$ C=O), 1678 ($\nu_{as}$ C=N), 1626 ($\nu_{as}$ $COO^-$), 1566 ($\delta$ $NH_2$), 1475 ($\delta$ $CH_2$), 1423 ($\nu_s$ $COO^-$), 1377 ($\delta$ CCH), and 1333 $cm^{-1}$ ($\gamma_w$ $CH_2$), where $\nu_{as}$, $\nu_s$, $\delta$, $\gamma_w$ indicate asymmetric stretching, symmetric stretching, in plane deformation and out-of-plane wagging, respectively (Krishnan and Sankaranarayanan, 1973). The glucose spectrum showed overlapping bands from about 1300 to 1460 $cm^{-1}$ corresponding to $CH_2$, CH and OH bending modes (Vazko et al., 1972). The spectra for Na-, Ca-, Al-, and Fe-clays + arginine + glucose systems in the 1300 to 1550 $cm^{-1}$ range had some similarities with the arginine spectrum. Peaks about 1475 and 1423 $cm^{-1}$ in the spectra for the treated clays are attributable to the $\delta$ $CH_2$ and $\nu_s$ $COO^-$, respectively. The $\nu$ C=O band (1720 $cm^{-1}$) of arginine was not evident in the spectra for the treated clays and the $\nu_{as}$ C=N and $\nu_{as}$ $COO^-$ bands were not resolved because of overlapping with the $\delta$ HOH of clays. At least two new peaks, 1650 and 1668 $cm^{-1}$, are evident in the spectra of the treated clays but not in the untreated clay or arginine spectra. The spectra for the Cu-saturated clays + arginine + glucose systems were different (1550 to 1300$cm^{-1}$ range) from the other spectra in that $\nu_s$ $COO^-$ band is shifted to a lower frequency (1388 $cm^{-1}$).

# DISCUSSION

The amount of C released as $CO_2$ during the incubations ranged from 0.0 to 2.5% of added C indicating that there was little or no microbial activity in the systems. Total C recoveries for the clay + arginine + glucose systems ranged from 81.4 to 123.8% of added C. Total C recoveries for two of the systems (Fe-SWa and Al-Panther) were less than 90% and total C recovery for the Fe-Otay was 123.8%, however recoveries for the other 15 systems, for which complete data sets were available, were between 90 and 110%. Thus in general we were able to account for all of the added C. Reasons for the two abnormally low and one abnormally high C recoveries are not clear. By contrast, total N recoveries averaged only 74.5% and ranged from 55.8 to 93.4% of added N. Nitrogen recoveries tended to be higher for the $Cu^{2+}$ systems and lower for the $Na^{+}$ and $Ca^{2+}$ systems. Thus we were not able to account for all of the added N. Volatilization loss of $NH_3$ gas, for systems with pH > 7.0, and loss of adsorbed arginine and $NH_4^{+}$ ions, when the clays were washed with 100 mM $CaCl_2$, are the most likely explanations for the incomplete N recoveries. For the clay systems (except the Cu-clays, which will be discussed separately) recoveries of soluble organic C from the solutions ranged from 42.1 to 90.0% of the total added C. The arginine-C + glucose-C in the solutions at the end of the incubations ranged from 28.8 to 64.4% of the total added C. Therefore, the data indicate that 7.1 to 33.7% of the added C was abiotically transformed to soluble compounds other than glucose and arginine during the incubations. The glucose-C to arginine-C ratio of the starting solutions was 1.00. By contrast, the glucose-C to arginine-C ratio of the solutions at the end of the incubations averaged 2.30 and ranged from 1.07 to 3.35. The

glucose-C to arginine-C ratio of the solutions for the Fe-treated clays was significantly lower than the ratios for the other cation treatments. These results indicate that arginine was either selectively sorbed on the clays and/or selectively decomposed in the solutions relative to glucose. High sorption of arginine on smectites has been reported (Dashman and Stotzky, 1982; Greenland et al., 1965; Hedges and Hare, 1987).

At the end of the 21-day incubations, between 14.9 and 50.3% of added C and between 16.5 and 90.0% of added N was sorbed on the clays in a form that could not be readily removed by washing with 100 mM $CaCl_2$ or distilled water. The mass C:N ratio of the adsorbed compounds ranged from 1.33 to 5.21, which is slightly to substantially higher than the mass C:N ratio of arginine (1.29) (Table 5) (except 1.16, 1.23, and 127 for Al-Saponite, Cu-Saponite and Cu-Otay, respectively). The mass C:N ratios for the sorbed compounds were significantly different among the cation treatments (Fe > Ca > Cu = Al) but no significant differences were observed for the various clays. The relatively high C:N ratios for the sorbed compounds indicate that some of the C originally in glucose was co-sorbed with the arginine-C on the clays. By contrast, analysis of glucose only (no arginine) interactions with the same clays under identical conditions (Chapter 3 of this dissertation) indicated that little or no glucose derived C was sorbed on the clays. The results from Chapter 3, however, do indicate that clays catalyze the dehydration of glucose to form HMF and levulinic acid under the conditions of this study. HMF and levulinic acid are highly reactive compounds capable of polymerizing or co-polymerizing with amino acids, Maillard reactions (Gogus et al., 1998).

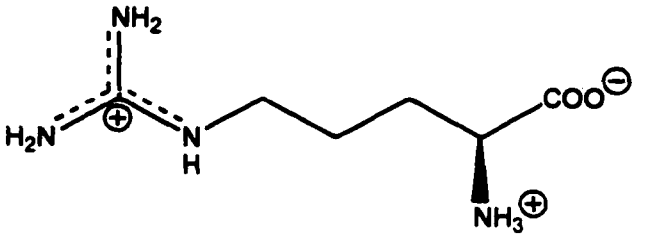
FT-IR spectra of the clay systems showed evidence that arginine and/or arginine + glucose condensation products interacted with the clay surfaces. The 1650 and 1668 $cm^{-1}$ bands, observed only for the clay systems (Fig. 2), can be assigned as stretching modes of amide group, quinones or C=O of H-bound conjugated ketones. Such functional groups are consistent with Maillard reaction products from clay + amino acid (glycine, tyrosine or tryptophan) + glucose systems (Arfaioli et al., 1997; Arfaioli et al., 1999; Bosetto et al., 2002; Bosetto et al., 1994). X-ray diffraction analysis indicated that the arginine + glucose treated clays had relatively consistent and stable *d*-spacing of about 1.4 nm (except the Fe-treated clays). *d*-spacing of 1.4 nm are consistent with a monolayer of organic molecules in the interlayers. Intercalation of arginine and other amino acids into the lamellar spaces of montmorillonite (*d*-spacing about 1.4 nm) have been reported (Dashman and Stotzky, 1985; Greenland et al., 1965; Naidja and Siffert, 1989). The *d*-spacing of the glucose + arginine treated Fe-clays were more variable probably due to the effects of inorganic $Fe_x(OH)_y$ interlayer polymers.

The high arginine-N sorption on Cu-Clays (84.0 to 90.0%) was observed and can be explained by the fact that amino acids form coordination complexes with $Cu^{2+}$ ions (de Farias et al., 2002; Weckhuysen et al., 1996). The FT-IR spectra of Cu-clays + arginine + glucose systems (Cu-Otay shown in Fig. 2) showed evidence of ligand exchange, the $\nu_s(COO^-)$ band shifted from 1423 to 1388 $cm^{-1}$ as reported for aqueous $Cu^{2+}$-arginine (Phan et al., 1975) and solid $Cu^{2+}$-arginine complexes (de Farias et al., 2002). Several other observations further support the formation of strong arginine-Cu-clay complexes; (1) $NH_4^+$-N from arginine dabsylation was small, 0.3 to

0.4% (Table 2b); (2) high sorbed C and N concentrations were observed; (3) the C:N ratio of the sorbed compounds, 1.3 to 1.4, was similar to that of arginine; and (4) only small amounts or arginine were detected in the solutions after the incubation (Table 1b). Moreover, the very high recovery of sorbed N for the Cu-clays (Table 2b) indicates that little of the sorbed arginine was desorbed when the samples were washed with 100 mM $CaCl_2$ and distilled water.

The goethites, by contrast with the smectites, sorbed relatively little glucose and arginine and were substantially less effective for catalyzing the transformation of glucose and arginine in the aqueous phase. Only a relatively small amount of C (6.2 to 9.0% of total added C) and even less N (2.3 to 4.6% of total added N) was sorbed on the goethites after the 21-day incubations. The ratio of glucose-C to arginine-C recovered in the aqueous solutions after the incubations ranged from 1.30 to 1.38, which is substantially lower than the glucose-C to arginine-C ratios for all of the smectite systems except the Fe-Panther. The results also indicate that the goethites were much less selective in the transformation and/or sorption of arginine relative to glucose than the smectites. There were no clear trends in the effects of degree of Al-substitution in the goethites on sorbed C or solution levels of glucose, arginine or $NH_4^+$. Minor differences in levels of sorbed N and total solution C are not consistent with the levels of Al-substitution.

Solution pH for the clay and goethite + arginine + glucose systems ranged from 4.3 to 8.9. Arginine is a basic amino acid with three $pK_a$'s; 2.01(COOH), 9.04 ($NH_2$), and 12.28 (guanidinium group):



Under the conditions of this study arginine is a zwitterions and sorption is not expected to be affected by solution pH. The Fe oxides, however, have a pH dependent surface charge with a point of zero charge (PZC) of about 8.3 (Herbillon, 1988). Because of the pH of the goethite systems (8.5 to 8.6) was near the PZC, relatively low sorption of arginine is anticipated.

Glucose is dehydrated under acidic conditions to form furfural compounds, whereas under alkaline conditions glucose is oxidized to form saccharinic acids (Theander and Nelson, 1978). Solution pH for the clay + arginine + glucose ranged from 4.3 to 8.9. Thus, it is possible that both reactions may have occured in the clay systems. It was reported in Chapter 3 of this dissertation that 5-(hydroxymethyl)-2-furfural (HMF), a Maillard reaction intermediate product, was formed from glucose dehydration and that HMF was further degraded to levulinic acid and formic acid. Although HMF and levulinic acid were not determined for the clay and goethite systems, it is expected that dehydration of glucose occurs under the conditions of the incubation. Amino acids have been shown to enhance the formation of HMF and melanoidins from a mixture of sugars (Gogus et al., 1998).

The ratio of $NH_4^+$-N relative to arginine-N in solution for all but three of the clay systems was higher (0.09 to 0.56) than the same ratio for the aqueous control (0.08). The high $NH_4^+$-N content relative to the control suggests that some arginine was

degraded during the incubation. Catalytic deamination and decarboxylation of amino acids by smectites (Naidja and Siffert, 1989; Siffert and Naidja, 1992) and by aldehydes and metal salts (Ikawa and Snell, 1954) have been reported.

New C and N are added to soil primarily as proteins and carbohydrates. During microbial degradation of proteins and carbohydrates, amino acids and monosaccharides are released to the soil solution. Although most of these labile compounds will be consumed by microbes, some will reach the surfaces of soil clays. The results of this study provide evidences that amino acids and monosaccharides are abiotically transformed by smectites. Furfural compounds and levulinic acid are the likely degradation products of monosaccharides. These compounds are known to be highly reactive, and thus are likely to be polymerized or co-polymerized with amino acids (Maillard reaction). Accordingly, the catalytic transformation of amino acids and monosaccharides by smectites and Fe oxides is suggested as one of the pathways for the incorporation of new C and N into SOM and for the formation of new humic substances. Fe oxides are less effective than smectites for catalyzing these reactions. Areas needing further investigation are the effects of pH, type of amino acid, and whether oligosaccharides and peptides are also catalytically transformed by smectites.

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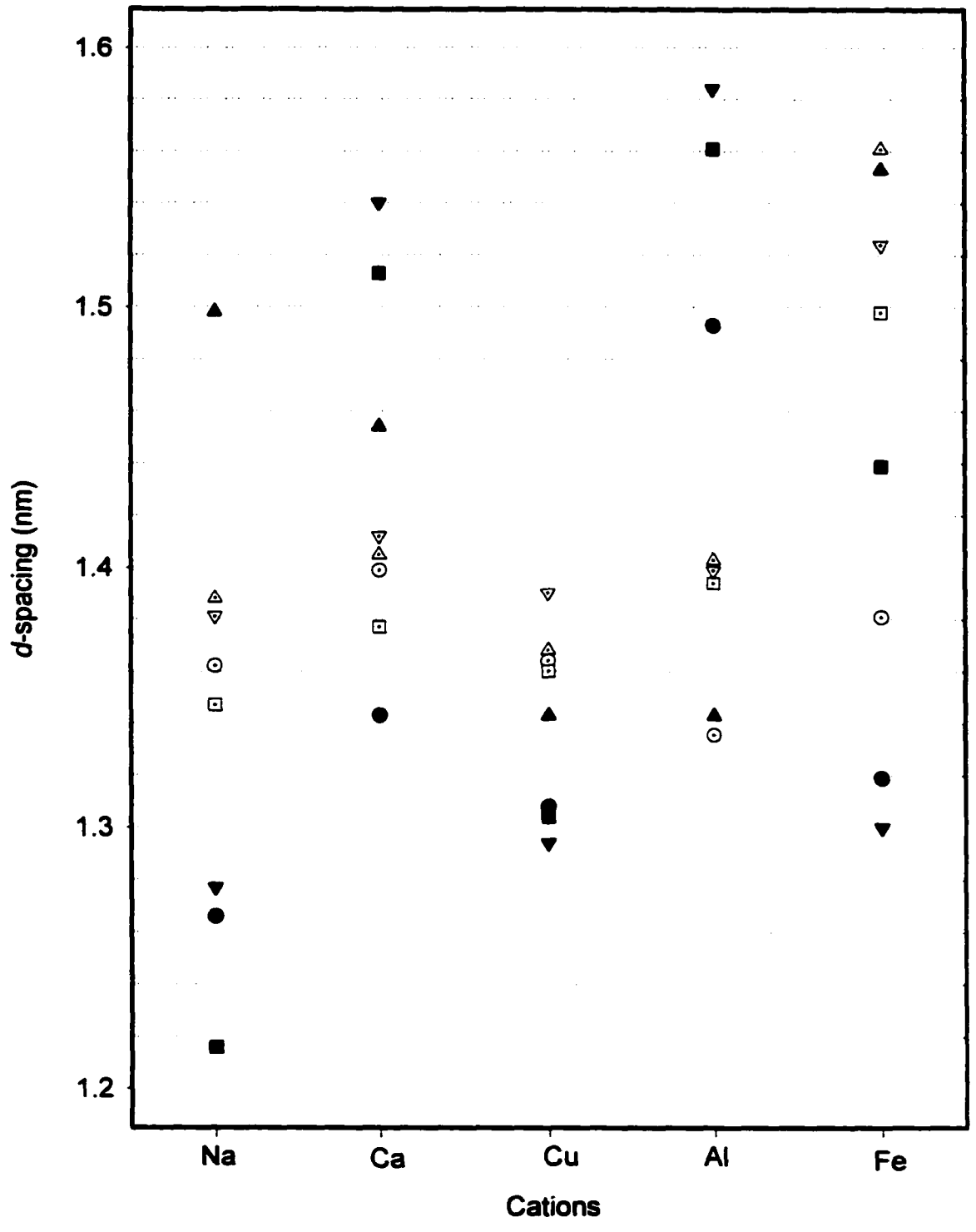


Figure 1. *d*-spacing for the samples of this study (filled circles represent untreated clays, open symbols represent treated clay with arginine and glucose; ● :SWa, ■ :Panther, ▲ :Saponite, ▼ :Otay).

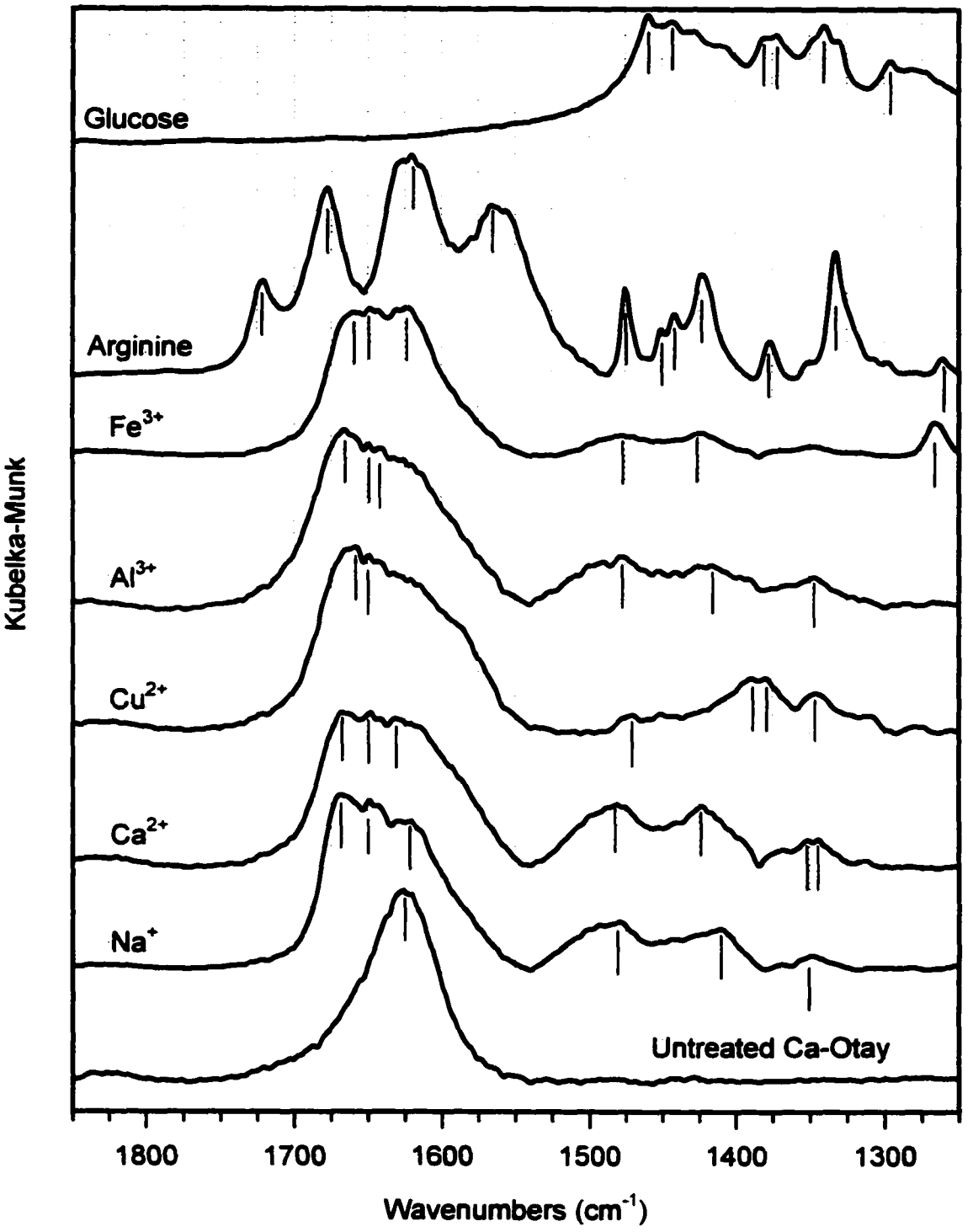


Figure 2. FT-IR spectra of the $M^{n+}$-Otay-Arginine-Glucose systems.

Table 1a. Mass balance of C for the clay + arginine + glucose systems.

| Sample | C in Solution | | | Sorbed C | $CO_2$-C | Total C |
|---|---|---|---|---|---|---|
| | Total† | Arginine-C# | Glucose-C# | | | |
| | % C Recovered | | | | | |
| ***SWa*** | | | | | | |
| Na- | 90.0 c§ | 14.4 b | 41.9 a | nd | 0.5 a | nd |
| Ca- | 69.4 b | 12.5 b | 27.1 a | 21.4 a | 0.1 a | 90.9 b |
| Cu- | 61.3 a | 1.6 a | 9.4 ¶ | 50.3 d | 0.0 a | 111.9 c |
| Al- | 84.0 c | 16.0 b | 35.2 a | 24.6 b | 0.1 a | 108.6 c |
| Fe- | 54.0 a | 13.0 b | 21.9 a | 26.8 c | 2.5 b | 81.4 a |
| ***Panther*** | | | | | | |
| Na- | 42.1 a | 13.2 c | 17.8 a | nd‡ | 1.5 ab | nd |
| Ca- | 70.8 b | 15.5 d | 48.2 a | 24.5 a | 0.0 a | 99.6 a |
| Cu- | 61.5 ab | 2.0 a | 23.1 | 45.0 b | 0.0 a | 106.6 a |
| Al- | 54.5 ab | 10.8 b | 35.5 a | 27.9 a | 0.1 a | 82.4 a |
| Fe- | 61.5 ab | 19.8 e | 21.2 a | 26.6 a | 1.9 b | 90.0 a |
| ***Saponite*** | | | | | | |
| Na- | 66.7 b | 13.2 b | 29.0 a | 38.8 d | 0.1 a | 106.4 a |
| Ca- | 77.5 cd | 15.7 b | 39.3 b | 21.7 a | 0.0 a | 99.2 a |
| Cu- | 59.5 a | 1.4 a | 39.2 | 47.7 e | 0.0 a | 107.3 a |
| Al- | 74.6 c | 16.2 b | 38.5 b | 26.1 b | 0.3 b | 101.1 a |
| Fe- | 80.6 d | 22.5 c | 40.6 b | 34.3 c | 0.3 b | 115.1 b |
| ***Otay*** | | | | | | |
| Na- | 66.2 a | 9.9 b | 18.9 a | 25.2 b | 0.0 a | 89.8 a |
| Ca- | 75.4 bc | 12.7 c | 38.2 b | 15.5 a | 0.0 a | 91.1 a |
| Cu- | 61.3 a | 0.9 a | 6.0 | 43.3 d | 0.0 a | 104.0 b |
| Al- | 73.6 b | 12.5 c | 41.9 b | 33.0 b | 0.0 a | 106.4 b |
| Fe- | 80.9 c | 22.2 d | 42.2 b | 42.6 d | 0.3 b | 123.8 c |

†: Determined by a modified dichromate oxidation method (Yeomans and Bremner, 1988).
‡ nd: not determined
§: Within each clay mineral and within each C-fraction, means followed by the same letter are not significant different by the Student-Newman-Keuls test ($P<0.05$).
¶ ¶ C-glucose for Cu-saturated clays was not used for the statistical analysis.
# C-Arginine and C-Glucose recoveries for controls (aqueous solution of arginine + glucose) were 44.7 and 38.9%, respectively.

Table 1b. Mass balance of C for the clay + arginine + glucose systems.

| Sample | C in Solution | | | Sorbed C | Total C |
|---|---|---|---|---|---|
| | Total† | Arginine-C# | Glucose-C# | | |
| | % C Recovered | | | | |
| ***Na*** | | | | | |
| SWa | 90.0 c§ | 14.4 a | 41.9 b | nd | nd |
| Panther | 42.1 a | 13.2 a | 17.8 a | nd§ | nd |
| Saponite | 66.7 b | 13.2 a | 29.0 a | 38.8 | 106.4 |
| Otay | 62.2 b | 9.9 a | 18.9 a | 25.2 | 89.8 |
| ***Ca*** | | | | | |
| SWa | 69.4 a | 12.5 a | 27.1 a | 21.4 b | 90.9 a |
| Panther | 70.8 a | 15.5 a | 48.2 c | 24.5 c | 99.6 a |
| Saponite | 77.5 a | 15.7 a | 39.3 b | 21.7 b | 99.2 a |
| Otay | 75.6 a | 12.7 a | 38.2 b | 15.5 a | 91.1 a |
| ***Cu*** | | | | | |
| SWa | 61.3 a | 1.6 b | 9.4 ¶ | 50.3 d | 111.9 c |
| Panther | 61.5 a | 2.0 c | 23.1 | 45.0 b | 106.6 b |
| Saponite | 59.5 a | 1.4 b | 39.2 | 47.7 c | 107.3 b |
| Otay | 61.3 a | 0.9 a | 6.0 | 43.3 a | 104.0 a |
| ***Al*** | | | | | |
| SWa | 84.0 c | 16.0 c | 35.7 a | 24.6 a | 108.6 c |
| Panther | 54.5 a | 10.8 a | 35.5 a | 27.9 a | 82.4 a |
| Saponite | 74.6 b | 16.2 c | 38.5 a | 26.1 a | 101.8 b |
| Otay | 73.4 b | 12.5 b | 41.9 a | 33.0 b | 106.4 c |
| ***Fe*** | | | | | |
| SWa | 54.0 a | 13.0 a | 21.9 a | 26.8 a | 81.4 a |
| Panther | 61.5 a | 19.8 a | 21.2 a | 26.6 a | 90.0 a |
| Saponite | 80.6 a | 22.5 a | 40.6 a | 34.3 a | 115.1 b |
| Otay | 80.9 a | 22.2 a | 42.2 a | 42.6 b | 123.8 b |

†: Determined by a modified dichromate oxidation method (Yeomans and Bremner, 1988).
‡ nd: not determined
§: Within each cation and within each C-fraction, means followed by the same letter are not significant different by the Student-Newman-Keuls test ($P<0.05$).
¶ C-glucose for Cu-saturated clays was not used for the statistical analysis.
# C-Arginine and C-Glucose recoveries for controls (aqueous solution of arginine + glucose) were 44.7 and 38.9%, respectively.

Table 2a. Mass balance of N for the clay + arginine + glucose systems.

| Sample | pH | N in Solution | | Sorbed N | Total N |
|---|---|---|---|---|---|
| | | Arginine-N# | $NH_4^+$-N | | |
| | | % N Recovered | | | |
| ***SWa*** | | | | | |
| Na- | 7.0 | 28.6 b‡ | 16.0 b | nd† | nd |
| Ca- | 7.2 | 24.8 b | 5.4 a | 30.2 b | 60.5 a |
| Cu- | 4.7 | 3.1 a | 0.4 a | 90.0 d | 93.4 b |
| Al- | 4.6 | 31.8 b | 2.0 a | 36.8 c | 70.6 a |
| Fe- | 6.8 | 25.8 b | 16.5 b | 20.8 a | 66.2 a |
| ***Panther*** | | | | | |
| Na- | 7.0 | 26.3 c | 9.3 b | nd | nd |
| Ca- | 6.5 | 31.0 d | 5.2 ab | 35.7 b | 71.8a |
| Cu- | 4.5 | 3.9 a | 0.4 a | 84.0 d | 88.3 b |
| Al- | 4.7 | 21.6 b | 1.2 a | 51.2 c | 73.9 a |
| Fe- | 7.0 | 39.3 e | 9.2 b | 21.4 a | 70.0 a |
| ***Saponite*** | | | | | |
| Na- | 7.0 | 26.3 b | 8.2 d | 16.5 a | 55.8 a |
| Ca- | 8.7 | 31.1 b | 5.2 c | 26.4 c | 62.8 b |
| Cu- | 5.6 | 2.9 a | 0.5 a | 86.9 e | 90.3 e |
| Al- | 6.4 | 32.3 b | 3.3 b | 46.3 d | 81.9 d |
| Fe- | 7.0 | 44.7 c | 4.6 bc | 22.4 b | 71.6 c |
| ***Otay*** | | | | | |
| Na- | 7.5 | 19.7 b | 6.2 d | 40.6 b | 67.3 b |
| Ca- | 8.9 | 25.4 c | 5.4 d | 27.8 a | 58.6 a |
| Cu- | 4.8 | 1.9 a | 0.3 a | 89.4 d | 91.6 c |
| Al- | 4.3 | 24.8 c | 1.2 b | 63.5 c | 89.5 c |
| Fe- | 7.0 | 44.3 d | 3.4 c | 29.3 a | 76.9 d |

†: nd: not determined

‡: Within each clay mineral and within each N-fraction, means followed by the same letter are not significant different by the Student-Newman-Keuls test ($P<0.05$).

# N-Arginine recovery for control (aqueous solution of arginine + glucose) was 88.9%

Table 2b. Mass balance of N for the clay + arginine + glucose systems.

| Sample | N in Solution | | Sorbed N | Total N |
|---|---|---|---|---|
| | Arginine-N | $NH_4^+$-N | | |
| | % N Recovered | | | |
| ***Na*** | | | | |
| SWa | 28.6 a‡ | 16.0 b | nd† | nd |
| Panther | 26.3 a | 9.3 a | nd | nd |
| Saponite | 26.3 a | 8.2 a | 16.5 a | 55.8 a |
| Otay | 19.7 a | 6.2 a | 40.6 b | 67.3 b |
| ***Ca*** | | | | |
| SWa | 24.8 a | 5.4 a | 30.2 c | 60.5 a |
| Panther | 31.0 a | 5.2 a | 35.7 d | 71.8 b |
| Saponite | 31.1 a | 5.2 a | 26.4 a | 62.8 a |
| Otay | 25.4 a | 5.4 a | 27.8 b | 58.6 a |
| ***Cu*** | | | | |
| SWa | 3.1 b | 0.4 b | 90.0 c | 93.4 b |
| Panther | 3.9 c | 0.4 b | 84.0 a | 88.3 a |
| Saponite | 2.9 b | 0.5 c | 86.9 b | 90.3 ab |
| Otay | 1.9 a | 0.3 a | 89.4 c | 91.6 ab |
| ***Al*** | | | | |
| SWa | 31.8 c | 2.0 ab | 36.8 a | 70.6 a |
| Panther | 21.6 a | 1.2 a | 51.2 c | 73.9 b |
| Saponite | 32.3 c | 3.3 b | 46.3 b | 81.9 c |
| Otay | 24.8 b | 1.2 a | 63.5 d | 89.5 d |
| ***Fe*** | | | | |
| SWa | 25.8 a | 16.5 a | 20.8 a | 66.2 a |
| Panther | 39.3 a | 9.2 a | 21.4 a | 70.0 a |
| Saponite | 44.7 a | 4.6 a | 22.4 a | 71.6 a |
| Otay | 44.3 a | 3.4 a | 29.3 b | 76.9 a |

†: nd: not determined

‡: Within each cation and within each N-fraction, means followed by the same letter are not significant different by the Student-Newman-Keuls test ($P<0.05$).

# N-arginine recovery for control (aqueous solution of arginine + glucose) was 88.9%

Table 3. Mass balance of C for the goethite + arginine + glucose systems.

| Sample | C in Solution | | | Sorbed C | $CO_2$-C | Total C |
|---|---|---|---|---|---|---|
| | Total† | Arginine-C | Glucose-C | | | |
| | % C Recovered | | | | | |
| Goe-00 | 98.3 c‡ | 29.3 a | 40.4 a | 6.2 a | 0.3 | 105.1 a |
| Goe-20 | 91.4 a | 30.1 a | 41.6 a | 8.1 a | 0.3 | 100.5 a |
| Goe-51 | 95.5 b | 28.8 a | 37.5 a | 8.8 a | 0.3 | 104.7 a |
| Goe-94 | 94.2 b | 29.5 a | 38.6 a | 9.0 a | 0.0 | 103.8 a |

†: Determined by a modified dichromate oxidation method (Yeomans and Bremner, 1988).
‡: Within each C-fraction, means followed by the same letter are not significant different by the Student-Newman-Keuls test ($P<0.05$).

Table 4. Mass balance of N for the goethite + arginine + glucose systems.

| Sample | pH | N-in Solution | | Sorbed N | Total N |
|---|---|---|---|---|---|
| | | Arginine-N | $NH_4^+$-N | | |
| | | % N Recovered | | | |
| Goe-00 | 8.5 | 58.2 a† | 7.7 a | 2.3 b | 67.5 a |
| Goe-20 | 8.6 | 59.9 a | 7.2 a | 3.5 a | 70.6 a |
| Goe-51 | 8.5 | 59.4 a | 7.4 a | 3.4 a | 69.1 a |
| Goe-94 | 8.5 | 58.8 a | 7.5 a | 4.6 c | 71.1 a |

†: Within each N-fraction, means followed by the same letter are not significant different by the Student-Newman-Keuls test ($P<0.05$).

Table 5. Sorbed C and N, and C:N ratios for the clay + arginine + glucose systems.

| Sample | C-Sorbed | N-Sorbed | C:N |
|---|---|---|---|
| | ——— *mg g$^{-1}$ clay* ——— | | |
| ***Na*** | | | |
| SWa | nd† | nd | nd |
| Panther | nd | nd | nd |
| Saponite | 24.0§ | 4.6 | 5.21 |
| Otay | 18.3 | 11.4 | 1.61 |
| ***Ca*** | | | |
| SWa | 13.6 a‡ | 8.6 b | 1.58 b |
| Panther | 16.9 b | 10.2 c | 1.66 b |
| Saponite | 12.0 a | 7.5 a | 1.60 b |
| Otay | 11.2 a | 7.8 a | 1.44 a |
| ***Cu*** | | | |
| SWa | 34.4 c | 25.6 b | 1.34 a |
| Panther | 31.2 ab | 23.5 a | 1.33 a |
| Saponite | 30.1 a | 24.6 b | 1.23 a |
| Otay | 32.1 b | 25.3 b | 1.27 a |
| ***Al*** | | | |
| SWa | 16.0 b | 10.5 a | 1.53 c |
| Panther | 19.1 c | 14.4 c | 1.33 b |
| Saponite | 15.2 a | 13.1 b | 1.16 a |
| Otay | 23.8 d | 17.7 d | 1.34 b |
| ***Fe*** | | | |
| SWa | 17.4 a | 6.0 a | 2.63 a |
| Panther | 18.8 a | 6.2 a | 3.02 a |
| Saponite | 21.1 b | 6.3 a | 3.35 a |
| Otay | 33.7 c | 9.0 b | 3.75 a |

†: nd: not determined

‡: Within each cation and within each sorbed fraction, means followed by the same letter are not significant different by the Student-Newman-Keuls test ($P<0.05$).

§ no statistical analysis for Na-clays because of few data

Table 5. Sorbed C and N, and C:N ratios for the clay + arginine + glucose systems.

| Sample | Sorbed C | Sorbed N | C:N |
|---|---|---|---|
| | ——— *mg g$^{-1}$ clay* ——— | | |
| ***Na*** | | | |
| SWa | nd† | nd | nd |
| Panther | nd | nd | nd |
| Saponite | 24.0§ | 4.6 | 5.21 |
| Otay | 18.3 | 11.4 | 1.61 |
| ***Ca*** | | | |
| SWa | 13.6 a‡ | 8.6 b | 1.58 b |
| Panther | 16.9 b | 10.2 c | 1.66 b |
| Saponite | 12.0 a | 7.5 a | 1.60 b |
| Otay | 11.2 a | 7.8 a | 1.44 a |
| ***Cu*** | | | |
| SWa | 34.4 c | 25.6 b | 1.34 a |
| Panther | 31.2 ab | 23.5 a | 1.33 a |
| Saponite | 30.1 a | 24.6 b | 1.23 a |
| Otay | 32.1 b | 25.3 b | 1.27 a |
| ***Al*** | | | |
| SWa | 16.0 b | 10.5 a | 1.53 c |
| Panther | 19.1 c | 14.4 c | 1.33 b |
| Saponite | 15.2 a | 13.1 b | 1.16 a |
| Otay | 23.8 d | 17.7 d | 1.34 b |
| ***Fe*** | | | |
| SWa | 17.4 a | 6.0 a | 2.63 a |
| Panther | 18.8 a | 6.2 a | 3.02 a |
| Saponite | 21.1 b | 6.3 a | 3.35 a |
| Otay | 33.7 c | 9.0 b | 3.75 a |

†: nd: not determined

‡: Within each cation and within each sorbed fraction, means followed by the same letter are not significant different by the Student-Newman-Keuls test ($P<0.05$).

§ no statistical analysis for Na-clays because of missing data

# CHAPTER 5

# ROLE OF IRON OXIDES ON THE CATALYTIC POLYMERIZATION OF GLYCINE PEPTIDES AND GLUCOSE

A paper to be submitted to Clays and Clay Minerals

Javier M. Gonzalez and David A. Laird

## ABSTRACT

Several theories have been proposed to explain the formation of humic-like substances, yet the theory that proposed the Maillard reaction condensation products as starting materials has not been explored under the conditions normally found in soils. Furthermore, there is lack of information of the role of Fe oxides on the formation of humic-like substances. The objectives of this study were to investigate the ability of Al-substituted goethites to abiotically catalyze the formation of humic-like materials using mixtures of glycine peptides and glucose under conditions normally found in the soil. Four synthetic Al-substitutes goethites were incubated with a mixture of glycine peptides and glucose solutions for 21 days at 37°C under abiotic conditions. All C added was recovered (100.5 to 105.3%). At the end the incubations, nearly 100% of the added C was accounted for as soluble C, whereas only 83.6 to 93.4% of the added C was peptide-C and glucose-C in the solutions, suggesting that 5.9 to

18.2% of the added C was abiotically transformed to other soluble organic compounds during the incubation by goethites. Only small amounts of sorbed C in the triglycine systems were found, from 3.8 to 4.8% of the added C. In the trigly systems, digly-N content was much higher than in the control, 4.9 to 10.0% and 0.1%, respectively, and digly-N increased as Al-substitution of goethites increased, suggesting that triglycine is cleaved by goethites and the reaction is likely affected by the level of Al substitution in goethites.

## INTRODUCTION

Soil organic matter (SOM) has many important roles in soils. SOM promotes the stabilization of soil structure; hence, improving aeration, water-holding capacity and permeability of soils. Likewise, mineralization of SOM releases N, P, S, and other nutrients that promote plant growth. The binding of organic pollutants and heavy metals to SOM reduces water contamination. Lately, C sequestration in SOM has been suggested as one possible means of reducing greenhouse gases in the atmosphere.

The fractionation of SOM yields humic and nonhumic substances. Humic substances are high-molecular-weight compounds formed from secondary synthesis reactions. Nonhumic substances consist of well-known biochemical compounds such as proteins, carbohydrates, lipids, etc. Although, the formation of SOM is not well understood, four major theories for the formation of humic substances have been proposed. The first theory proposes that lignin is used by microorganisms and that modified lignin residue becomes part of the SOM. The second theory hypothesizes

that the byproducts of lignin (phenolic compounds and organic acids) are condensed and polymerized to form humic substances (Stevenson, 1982; Stevenson and Cole, 1999). The third theory also hypothesizes that polyphenols are the building blocks of humic substances; however, the polyphenols are hypothesized to be microbial transformation products from non-lignin C sources. These polyphenols are oxidized to quinones and then co-polymerize with other compounds to form humic substances (Stevenson, 1982; Stevenson and Cole, 1999). The fourth theory is that Maillard reactions products are the building blocks of SOM (Stevenson, 1982; Stevenson and Cole, 1999).

Both biotic and abiotic catalytic polymerization of polyphenols has been widely studied. Microorganisms are capable of transforming phenols into humic-like substances (Haider and Martin, 1970; Martin et al., 1972; Pal et al., 1994; Saiz-Jimenez et al., 1975; Sulfita and Bollag, 1981). It has been reported that enzyme-catalyzed reactions (laccase, *p*-diphenol oxidase from fungi) are more effective for oxidizing phenols than abiotic-catalyzed reactions with birnessite, a Mn oxide (Pal et al., 1994). Clay minerals and various Fe, Al and Mn oxides have been reported to serve as catalysts for the formation of humic-like substances from mixtures of phenols and glycine (Lehmanh et al., 1987; Naidja et al., 1998; Wang, 1991; Wang and Huang, 1989; Wang and Huang, 1991; Wang, 1993; Wang, 1987; Wang et al., 1978; Wang et al., 1983).

The Maillard reaction is the non-enzymatic condensation of amino acids and reducing sugars to form melanoidins (insoluble brown nitrogenous containing compounds). Clay minerals can abiotically catalyzed the Maillard reaction when

samples are subjected to "wetting-drying" cycles at relative high temperatures (70 to 100 °C) (Arfaioli et al., 1997; Arfaioli et al., 1999; Bosetto et al., 2002). There is vast amount of information on the role of clay minerals in catalyzing melanoidins condensation; however, most of the studies have been designed to mimic pre-biotic conditions on earth.

Monosaccharides transformation and co-polymerization with amino acids by smectites and iron oxides under conditions similar to those normally found in soils have been reported previously (Chapters 3 and 4 of this dissertation). Smectites have greater capacity than iron oxides to transform glucose into furfural compounds and levulinic acid in systems containing only clay (or iron oxide) + glucose (Chapter 3). Similar results were obtained when the smectites or iron oxides + arginine + glucose were incubated under similar conditions. In addition to glucose transformation, arginine transformation was assumed for both clay and iron oxides systems since total N recovery was lower than the no-clay (or no-iron oxide) + arginine + glucose systems (Chapter 4). Furthermore, it has been reported that clay minerals and oxides of Al and Si abiotically catalyze peptide bond formation in systems containing clay minerals (or oxides of Al or Si) and alanine and/or glycine peptides (Bujdák and Rode, 1996; Bujdák and Rode, 1997; Bujdák and Rode, 1999; Bujdák et al., 1996; Bujdák et al., 1994; Bujdák et al., 1995). Also, it has been reported that transition metals catalyze the Maillard reaction of proteins and glucose (Hayase et al., 1996). However, there is lack of information on the ability Fe oxides to abiotically catalyze the condensation of small peptides and reducing sugars.

The specific objective of this study was to determine whether goethites

catalyze the abiotic co-polymerization of glycine peptides and glucose to form humic-like compounds.

## MATERIALS AND METHODS

### Materials

Chemicals: L-glycylglycine (digly) and L-glycylglycylglycine (trigly) (>98% purity) and ACS reagent grade D-glucose (Sigma-Aldrich Corp., St. Louis, MO) were used without further purification. DABS-Cl [4-(Dimethylamino)azobenzene-4-sulfonyl chloride] (96% purity) (Sigma-Aldrich Group, St. Louis, MO) was used for the pre-column derivatization of digly, trigly, and glucose. Milli-Q water (Milli-Q system, Millipore, Bedford, MA) with a resistivity of 18.2 MΩ-cm was used to prepare all solutions.

### Samples preparation

Sample preparation has been described in Chapter 3 of this dissertation. Briefly, four Al-substituted goethites with different Al/(Fe+Al) mol/mol ratios were synthesized (Schwertmann and Cornell, 2000). Mixtures with 0, 20, 50 and 120 mL of 0.3125 M of aluminate solution and 180, 178, 174, and 165 mL, respectively, of 5.0 M KOH were prepared in polyethylene containers. Then, 100 mL of freshly prepared 1.0 M $Fe(NO_3)_3$ solution were added quickly to each container, the samples were diluted to 2 L with distilled water, mixed and placed in the oven for 14 days at 70°C. After crystallization, the samples were centrifuged to separated the precipitated, and the precipitate was washed twice with 400 mL 1.0 M KOH to remove excess Al. Then

the pH was adjusted to 7.5 with HCl, and the samples were washed once with distilled water, and freeze dried.

**Incubation**

About 0.50 g of Al-substituted goethite was placed in 15-mL amber vials with open-top phenolic screw caps and loosely capped using PTFE/silicone septa (Supelco, Bellefonte, PA). The capped vials were sterilized in an autoclave for 15 min at 120 °C. The digly and trigly (called collectively peptides in this chapter) and glucose solutions were prepared fresh for each experiment, the solutions were filter-sterilized with 150-mL Nalgene® MF75 Series sterile disposable tissue culture filter units (Nalge Nunc International, Rochester, NY) and added to the cooled-vials containing the sterilized Al-substituted goethite under sterile conditions to avoid microbial contamination. The final concentration of peptide and glucose was about 0.5 mmol $g^{-1}$-goethite each or combined total of 1.0 mmol $g^{-1}$ goethite of both compounds in the system. The total solution volume was 5 mL. The solutions were dispended using a Repeater® Plus pipet with autoclave-sterilized 50-mL Eppendorf Combitip Plus tips (Eppendorf AG, Hamburg, Germany). The vials containing the Al-substituted goethite + peptide + glucose systems were capped tightly and a strip of wax paper was wrapped around the caps to further insure the integrity of the systems. The vials were vortex-mixed and incubated in the dark for 21 days in a temperature-controlled incubator at 37±0.5°C. Three replicates were run for each treatment.

**Analysis**

After incubation, the evolved $CO_2$ concentration was measured with a $CO_2$ infrared gas analyzer (for details, refer to Chapter 3 of this dissertation). After the $CO_2$ analysis, 5-mL of a 100 mM filter-sterilized $CaCl_2$ solution were added to the vials under sterile conditions, mixed and centrifuged for 10 min at 4500 x *g*. The supernatant was analyzed for the peptide, glucose and total organic C, and the pH of the supernatant was measured.

Peptide analysis was performed by reverse phase-HPLC using the pre-column derivatization or "dabsylation" (refer to Chapter 4 for more details). Briefly, a 25-μL sample aliquot, 500 μL of 50 mM $NaHCO_3$ buffer solution (pH 9.0) and 750 μL of 4 mM DABS-Cl solution were added to a clear 2-mL screw cap sample vial. The vials were capped and the samples were reacted for 30 min in a temperature-controlled block heater set at 70°C. A reverse phase ODS $C_{18}$ column (4.6 by 150 mm, particle size5 μm) was used. The injection was performed using a 10-μL loop syringe loading injector, and absorbance was measured at 470 nm. The mobile phase for the analysis of peptides consisted of acetonitrile (A) and a 40/60 v/v mixture of acetonitrile/water (pH 3.0) (B). A flow rate of 0.8 mL $min^{-1}$ was used with the following gradient profile: 0 min 100% B; 2.0 min 100% B; 7.0 min 10% B; 13 min 10% B; and 3 min re-equilibration with 100% B. The retention times for trigly and digly were 4.7 and 5.9 min, respectively. Calibration was performed using external standards. Elution of excess DABS-Cl was observed at 2.6 min. Dabsylated $NH_4^+$ and glycine were also quantified using external standards.

Glucose analysis has been described in Chapter 3 of this dissertation. Briefly,

a 25-μL aliquot of supernatant was placed in a 2-mL clear vial and dried under a stream of $N_2$ gas in a sand bath at 40°C. To the dried sample, 400- μL of a DABS-hydrazine solution was added, the vials were capped and the samples reacted for 30 min at 70°C. The solution was allowed to cool to room temperature and immediately analyzed by reverse phase-HPLC. The mobile phases consisted of acetonitrile (A) and a mixture (40/60 v/v) of acetonitrile/water (pH 3.0) (B), with a flow rate of 0.8 mL $min^{-1}$. The following gradient profile was used: 0 min 100% B; 6.0 min 100% B; 10 min 10% B; 15 min 10% B, and 5 min with re-equilibration with 100% B. The retention time for glucose was 5.6 min. Calibration was performed using external standards. Peaks for the un-reacted DABS-Cl and DABS-hydrazine were observed at 3.0 and 14 min, respectively.

Organic carbon analysis in the supernatant was described in Chapter 3 of this dissertation. Briefly, an aliquot of 2.0 mL of supernatant, 5.0 mL of 1 N $K_2Cr_2O_7$ and 7.5 mL of concentrated $H_2SO_4$ were added to an Erlenmeyer flask. The flask was placed on a hot plate, connected to a condenser and allowed to boil for 15 minutes. The flasks were allowed to cool to room temperature, 0.3 mL of indicator solution was added, and the samples were titrated with Mohr's salt solution. Two boiled and two unboiled controls were also prepared.

After the analysis of peptides, glucose and total organic C, and pH measurements were complete; the supernatants for the trigly systems were decanted. To remove excess salt and organic compounds, the suspension was rinsed with water and centrifuged for 10 min at 4000 x *g* several times until the conductivity of the supernatant was <0.6 mS/m. Then, the samples were freeze-dried. The total C and

total N in the freeze-dried samples were determined by dry combustion using a Carlo-Erba NA1500 NSC elemental analyzer (Haake Buchler Instruments, Paterson, NJ). X-ray diffraction (XRD) patterns for the freeze-dried treated and untreated samples were obtained using a Scintag XDS-2000 powder diffractometer (Thermo ARL, Ecublens, Switzerland) equipped with an air-cooled Kevex Psi Peltier Silicon detector, and CuKα radiation. For the infrared spectra, the Diffuse Reflectance Infrared Fourier Transformed (DRIFT) technique in a Nicolet model Magna 500 equipped with a DTGS detector (Madison, WI) was used (for details, see Chapters 3 and 4 of this dissertation). One Way Analysis of Variance (ANOVA) and pairwise multiple comparison procedures using the Student-Newman-Keuls Method ($P<0.05$) were performed with the SigmaStat software (SPSS Science, Chicago, IL).

## RESULTS

### Characterization of the Al-substituted goethites

The four Al-substituted goethites used in this study were described in Chapter 3 of this dissertation. In summary, Al-substituted goethites were named Goe-00, Goe-20, Goe-51, and Goe-94 after their level of Al-substitution (0, 2.0, 5.1, and 9.4% mol/mol Al /(Al+Fe), respectively). All four samples showed typical goethite XRD reflections *(hkl)*:*110, 120, 130, 111, 140, 021, 101, 040* and *121*. FT-IR spectra for the Al-substituted goethites showed that the Fe-O-H deformation bands were 892, 902, 905, and 911 $cm^{-1}$ for the Goe-00, -20, -51, and -94, respectively. The total C content ranged from 0.10 to 0.15% and the total N content was negligible.

**C mass balance**

Carbon mass balances for the digly and trigly systems are presented in Tables 1 and 3, respectively. From 100.6 to 105.3% of the total C added was recovered for all systems. For the digly systems, digly-C and glucose-C in solution ranged from 28.2 to 37.5% and from 51.4 to 58.3% of the total C added, respectively. Glycine-C in the solutions ranged from 0.8 to 1.0% and evolved $CO_2$-C ranged from 0.0 to 0.9% of the total C added. For the trigly systems (Table 3), total soluble organic C ranged from 96.5 to 100.7% and trigly-C and glucose-C in solution ranged from 36.2 to 41.9% and 45.3 to 50.8%, respectively. For the trigly systems, degradation of trigly occurred to yield digly-C (2.5 to 5.0%), glycine-C (0.6 to 1.0%) and $CO_2$-C (0.0 to 0.7%). Only small amounts of C were sorbed on the goethites, from 3.8 to 4.6% of the total added C. The recoveries for no-goethite controls (digly + glucose and trigly + glucose) were 95.4 and 104.1%, respectively.

**N mass balance**

Nitrogen mass balance for the Al-substituted goethite + peptide + glucose systems are presented in Tables 2 and 4. For the digly systems (Table 2), total recovered N ranged from 91.1% to 99.0% and digly-N recoveries were 85.3 to 93.8%, except for the Goe-20 systems. N-recovery and digly-N recovery were only 75.4 and 70.4%, respectively. Small amounts of glycine-N, and $NH_4^+$-N (1.9 to 2.4 and 2.8 to 4.4%, respectively) were recovered from the digly systems. High recoveries of total N and trigly-N were obtained for the trigly systems, from 90.8 to 97.1% and 72.5 to 83.8% of the total N-added, respectively (Table 4). Relative high levels of digly-N (4.9

to 10.0%) and lower levels of glycine-N and $NH_4^+$-N (1.1 to 2.1% and 1.6 to 3.4%, respectively) were found in the trigly systems. Only, small amounts of sorbed N (1.4 to 3.0%) were found.

**FT-IR and XRD**

FT-IR spectra and XRD patterns for the trigly systems (not shown) were similar to those of the untreated goethites. No relevant absorption bands for organic compounds were observed.

## DISCUSSION

Small amounts of evolved $CO_2$ during the incubations (0 to 0.9% of total added C) indicate that there was little or no microbial activity in the systems. Complete C recoveries for all systems were obtained (from 100.6 to 105.3%). Likewise, nearly all N was recovered, from 90.8 to 99.0% from all systems except the Goe-20 + dilgy + glucose systems (75.4%). The most likely explanations for the incomplete N recoveries is the accumulation of $NH_4^+$ due to the peptide bond cleavage during the incubation experiments. The reasons for the unusually low recoveries of total N and digly-N for the Goe-20 + digly + glucose systems (75.4 and 70.4%, respectively) are not clear.

At the end of the incubations, the soluble organic C recovered from both digly and trigly systems ranged from 97.9 to 103.0% of the added C, whereas, the sum of peptides-C and glucose-C in the solutions ranged from 83.6 to 94.6% of the added C. Thus, the data suggest that from 5.9 to 18.2% of the added C was abiotically transformed to unknown soluble compounds during the incubations. For the digly

systems, the glucose-C to digly-C ratio on day-0 of the incubations was 1.50. At the end of the incubations, the glucose-C to digly-C ratio for Goe-00, -20, -51, and -94 systems was 1.70, 1.93, 1.50, and 1.46, respectively. For the trigly systems the glucose-C to trigly-C ratio was 1.00 on day-0 of the incubations and 1.10 to 1.40 at the end of the incubations. These results suggest that digly was selectively transformed in the solutions relative to glucose in the Goe-00 and Goe-20 systems and that trigly was selectively sorbed on the Fe oxides and/or selectively transformed in the solutions relative to glucose in all of the goethite systems.

At the end of the incubations, from 3.8 to 4.8% of added C and from 1.4 to 3.0% of added N could not be desorbed from the goethites with $CaCl_2$ or distilled water in the trigly systems. Sorbed C and N were not determined for the digly systems but are assume to be negligible because total recoveries from the solution exceeded 100% of the added C and 90% for added N (except total N for Goe-20). The relatively low sorption of peptides in these systems is likely due to the pH effect. At the solution pH's (5.1 to 5.2), both goethites and peptides have a net positive charge. The mass C:N ratios of the sorbed compounds were considerably higher (from 4.82 to 11.17) than the mass C:N ratio of trigly (1.71). Only the mass C:N ratios of Goe-00 were significant different from the other goethites. Thus, the above results suggest that C-rich compounds, glucose and/or glucose derivatives were co-adsorbed with the peptides on the goethites. By contrast, data for the glucose-goethite interactions (Chapter 3 of this dissertation) indicated that little or no glucose derived C was sorbed on goethites when no peptides were present. The results of Chapter 3 of this dissertation indicate that glucose is dehydrated by goethites to form

5-(hydroxymethyl)-2-furfural (HMF) and levulinic acid under similar conditions of this study. HMF and levulinic acid are highly reactive compounds capable of polymerizing or co-polymerizing with amino acids (Maillard reaction). It has been reported that amino acids enhance the formation of HMF and melanoidins from a mixture of sugars (Gogus et al., 1998; Reyes et al., 1982). The XRD patterns and the FT-IR spectra of the trigly systems did not show differences from the untreated goethites, suggesting that the compounds were sorbed on external surfaces and edges of the Fe oxides.

Higher digly-N contents in solution were found in trigly systems than in controls, 4.9 to 10.0% and 0.1%, respectively (Table 4). Also, it appears that digly-N increases as the level of Al substitution increases in goethites. Similar trend is observed for the digly-C (Table 3). Thus, these results indicate contrary to that observed for smectite, alumina and silica + peptide systems under pre-biotic conditions (Bujdák and Rode, 1996; Bujdák and Rode, 1997; Bujdák and Rode, 1999; Bujdák and Rode, 2002), that Fe oxides cleaved the peptide bond of trigly under the conditions of this experiment.

During the degradation of plant and animal material, new C and N are added to soils primary as proteineous material and carbohydrates. Degradation of proteineous materials and carbohydrates releases peptides, amino acids and carbohydrates. Some of these compounds will reach surfaces of the Fe oxides in soils. The results of this study did not provide clear evidences for the Maillard reaction of peptides and monosaccharides. However, the results provided evidence that iron oxides cleaved the peptides bond of trigly and that the extent of the cleavage increases as Al substitution increases in goethites. Accordingly, the abiotically catalytic cleavage of peptide bonds by iron oxides is suggested that as one of the pathways for the

degradation of peptides in soil environments.

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Table 1. Mass balance of C for the Al-substituted goethite + digly + glucose systems.

| Sample | C in Solution | | | | $CO_2$-C | Total C |
|---|---|---|---|---|---|---|
| | Total† | Digly-C | Glycine-C | Glucose-C | | |
| | % C Recovered | | | | | |
| Goe-00 | 102.9 a‡ | 34.1 b | 0.8 a | 58.3 b | 0.9 | 104.2 b |
| Goe-20 | 101.9 a | 28.2 a | 0.9 a | 54.5 b | 0.7 | 102.1 ab |
| Goe-51 | 103.0 a | 37.5 b | 0.7 a | 56.2 b | 0.7 | 103.0 b |
| Goe-94 | 100.6 a | 35.1 b | 1.0 a | 51.4 a | 0.0 | 101.2 a |
| Control# | nd§ | 37.2 b | 1.0 a | 57.8 b | 0.0 | 96.0¶ c |

†: Determined by a modified dichromate oxidation method (Yeomans and Bremner, 1988).
‡: Within each C fraction, means followed by the same letter are not significantly different by the Student-Newman-Keuls test ($P<0.05$).
§: not determined
¶: sum of digly-C, glycine-C and glucose-C
#: equalmolar solutions of trigly and glucose

Table 2. Mass balance of N for the Al-substituted goethite + digly + glucose systems.

| Sample | pH | N in Solution | | | Total N |
|---|---|---|---|---|---|
| | | Digly-N | Glycine-N | $NH_4^+$-N | |
| | | % N Recovered | | | |
| Goe-00 | 4.9 | 85.3 a† | 2.0 a | 3.8 b | 91.1 a |
| Goe-20 | 5.0 | 70.4 a | 2.2 a | 2.8 a | 75.4 a |
| Goe-51 | 5.0 | 93.8 a | 1.9 a | 3.3 a | 99.0 a |
| Goe-94 | 4.9 | 87.7 a | 2.4 a | 4.4 c | 94.5 a |
| Control‡ | 6.2 | 93.1 a | 2.1 a | 2.8 a | 98.0 a |

†: Within each N fraction, means followed by the same letter are not significantly different by the Student-Newman-Keuls test ($P<0.05$).
‡: equalmolar solutions of trigly and glucose

Table 3. Mass balance of C for the Al-substituted goethite + trigly + glucose systems.

| Sample | C in Solution | | | | | Sorbed C | $CO_2$-C | Total C |
|---|---|---|---|---|---|---|---|---|
| | Total† | Trigly-C | Digly-C | Glycine-C | Glucose-C | | | |
| | % C Recovered | | | | | | | |
| Goe-00 | 97.9 ab‡ | 40.5 a | 2.5 b | 0.6 a | 48.2 a | 4.6 a | 0.7 | 102.8 a |
| Goe-20 | 100.0 b | 41.9 a | 3.8 c | 0.7 b | 46.1 a | 4.8 a | 0.6 | 105.3 a |
| Goe-51 | 100.7 b | 41.1 a | 4.2 c | 0.8 b | 45.3 a | 3.8 a | 0.5 | 105.0 a |
| Goe-94 | 96.5 a | 36.2 a | 5.0 c | 1.0 c | 50.8 a | 4.2 a | 0.0 | 100.6 a |
| Control¶ | nd§ | 56.1 b | 0.1 a | 1.7 a | 46.3 a | nd | 0.0 | 104.1# a |

†: Determined by a modified dichromate oxidation method (Yeomans and Bremner, 1988).
‡: Within each C fraction, means followed by the same letter are not significantly different by the Student-Newman-Keuls test (P<0.05).
§: not determined
¶: equalmolar solutions of trigly and glucose
#: sum of trigly-C, digly-C, glycine-C and glucose-C

Table 4. Mass balance of N for the Al-substituted goethite + trigly + glucose systems.

| Sample | pH | N in Solution | | | | Sorbed N | Total N |
|---|---|---|---|---|---|---|---|
| | | Trigly-N | Digly-N | Glycine-N | $NH_4^+$-N | | |
| | | % N Recovered | | | | | |
| Goe-00 | 5.2 | 81.0 a† | 4.9 b | 1.1 a | 2.8 a | 1.4 a | 90.8 a |
| Goe-20 | 5.1 | 83.8 a | 7.6 c | 1.5 a | 2.7 a | 2.3 c | 97.1 a |
| Goe-51 | 5.1 | 82.2 a | 8.4 c | 1.5 a | 1.6 a | 1.8 b | 94.9 a |
| Goe-94 | 5.1 | 72.5 a | 10.0 c | 2.1 b | 3.4 a | 3.0 d | 91.0 a |
| Control‡ | 5.5 | 112.1 b | 0.1 a | 3.4 c | 6.1 b | nd§ | 121.7 b |

†: Within each N fraction, means followed by the same letter are not significantly different by the Student-Newman-Keuls test (P<0.05).
‡: equalmolar solutions of trigly and glucose
§: not determined

# CHAPTER 6

# GENERAL CONCLUSIONS

The formation and stabilization of soil organic matter (SOM) is not well understood. The Maillard reaction has been proposed as an alternative theory for SOM formation. Maillard reactions have been extensively studied under pre-biotic conditions, but not under conditions relevant for SOM formation. Furthermore, it is well-known that clay minerals catalyze various organic reactions. Thus, we hypothesized that clay minerals abiotically catalyze the Maillard reaction under soil conditions. The specific objectives of this dissertation were: 1) to study the distribution of newly formed humic materials into mineralogical distinct clay-size fractions of a silt loam soil; 2) to determine whether smectites (type of smectite and their associated saturating metal cations) and synthetic Al-substituted goethites catalyze the abiotic polymerization of glucose to form humic-like compounds; 3) to determine whether smectites (type of smectite and their associated saturating metal cations) and synthetic Al-substituted goethites catalyze the abiotic co-polymerization of arginine and glucose to form humic-like compounds; and 4) to determine whether synthetic Al-substituted goethites catalyze the abiotic co-polymerization of glycine peptides and glucose to form humic-like compounds.

In Chapter 2 the distribution newly formed humic substances among mineralogically significant coarse, medium, and fine clay sized fractions was investigated. The study showed that the fine clay fraction was dominated by an

interstratified smectite/illite phase, whereas the coarse clay fraction was dominated by quartz with lesser amounts of kaolinite, illite, smectite and feldspars. The total organic C and total N content in the fine and coarse clay fractions were similar. However, the new $^{14}C$ preferentially accumulated in the fine clay fraction relative to the coarse clay fraction. SEM's reveal that diffuse humic materials exist on the surfaces of 2:1 phyllosilicates in the fine clay fractions, whereas discrete C rich particles in the coarse clay fraction were assumed. Thus, these results suggest that new humic materials are preferentially forming or accumulating on soil smectite surface.

In the third Chapter, the role of clay minerals and iron oxides on the abiotic transformation of organic compounds of biochemical importance were studied under conditions similar to those found in soils. Because carbohydrates are major components of the organic residues added to soils, the ability of clay minerals and iron oxides to catalyze transformations of monosaccharides was studied. The results showed that smectites and to a lesser extent iron oxides were capable of catalyzing dehydration of monosaccharides to form furfural compounds and levulinic acid. Also, monosaccharide dehydration was affected by the type of saturating cation on the clay; $Al^{3+}$ enhanced monosaccharide dehydration whereas $Fe^{3+}$ inhibited monosaccharide dehydration.

Amino acids and peptides are also major components of plant residues. Thus, the abiotic co-polymerization of monosaccharides and amino containing compounds by clay minerals and iron oxides were studied in Chapters four and five. The results provided evidences that amino acids and monosaccharides are abiotically

transformed more efficiently by smectites than iron oxides. Small glycine peptides and glucose were abiotically transformed by synthetic Al-substituted goethites. In iron oxides systems, as Al substitution on goethites increases, cleavage of the peptide also increases. The transformation products in the systems (sorbed C + N) are believed to be co-polymerization products of furfural compounds and levulinic acid and amino containing compounds.

The hypothesis that Maillard reactions contribute to the formation of the new humic substances has been considered for many years. However, the hypothesis has been largely discounted because the kinetics of glucose dehydration in soil environments was believed to be very slow. A key finding of this study is that clay minerals are capable of catalyzing the dehydration of glucose under conditions found in soils. Therefore, the study demonstrates that Maillard reactions may be a major pathway for the incorporation of new C and N into SOM and for the formation of new humic substances. Areas needing further investigation are the effects of pH, type of amino acid, and whether oligosaccharides and peptides are also catalytically transformed by smectites and iron oxides.

# APPENDIX A

## RAW DATA FROM CHAPTER 2

(p. 144-152)

The appendix consists of four sections. Each section consists of the raw data from each chapter.

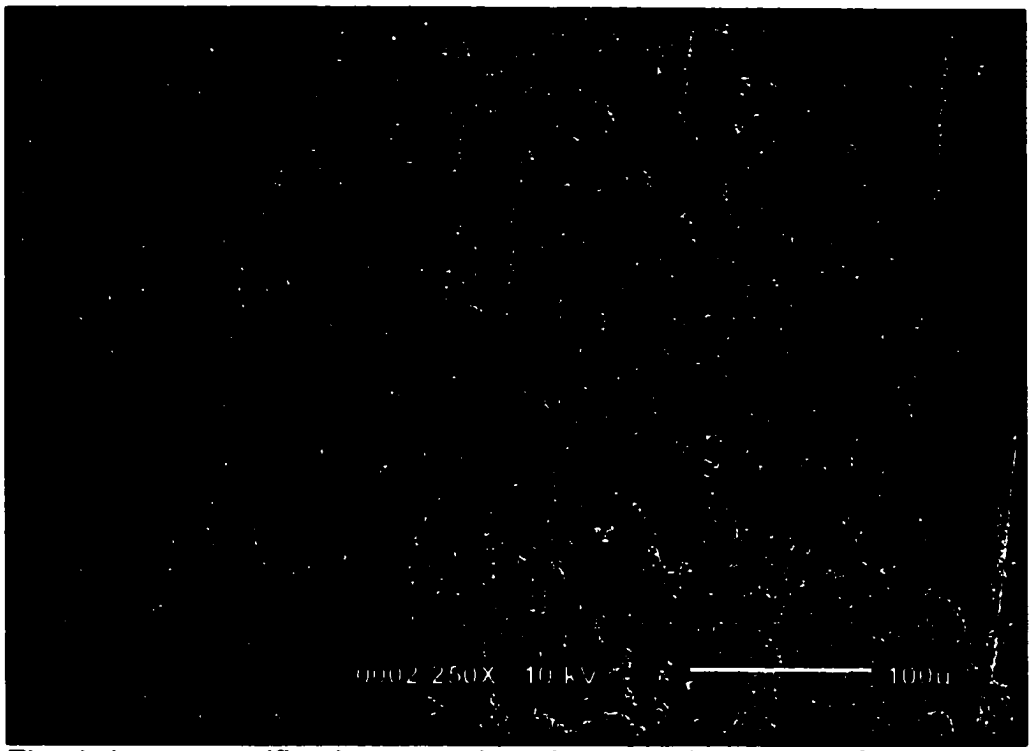

Fig. 1. Low magnification-scanning electron micrograph of the whole clay fraction from a Monona soil.

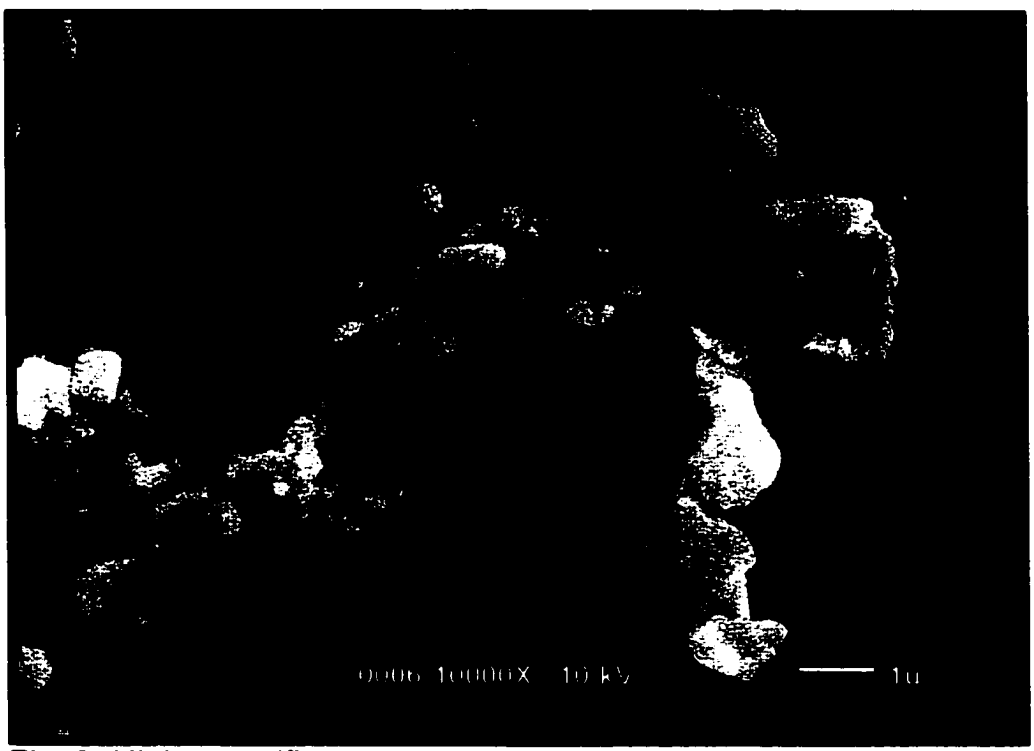

Fig. 2. High magnification-scanning electron micrograph of the medium clay fraction from a Monona soil.

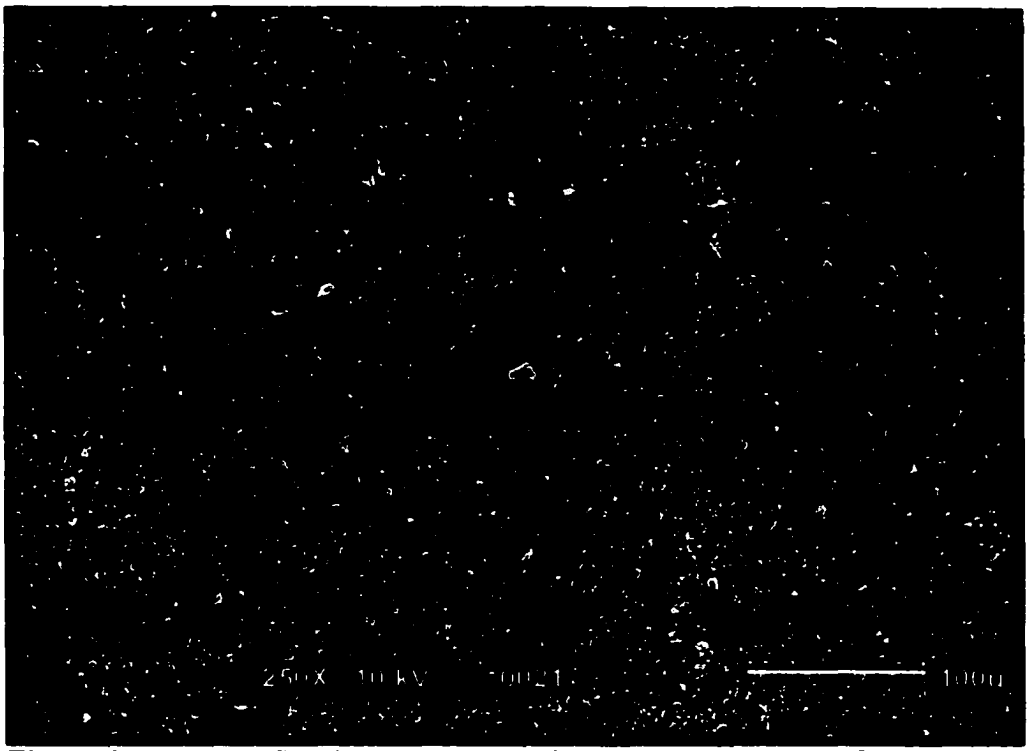

Fig. 3. Low magnification-scanning electron micrograph of the coarse clay fraction from a Monona soil.

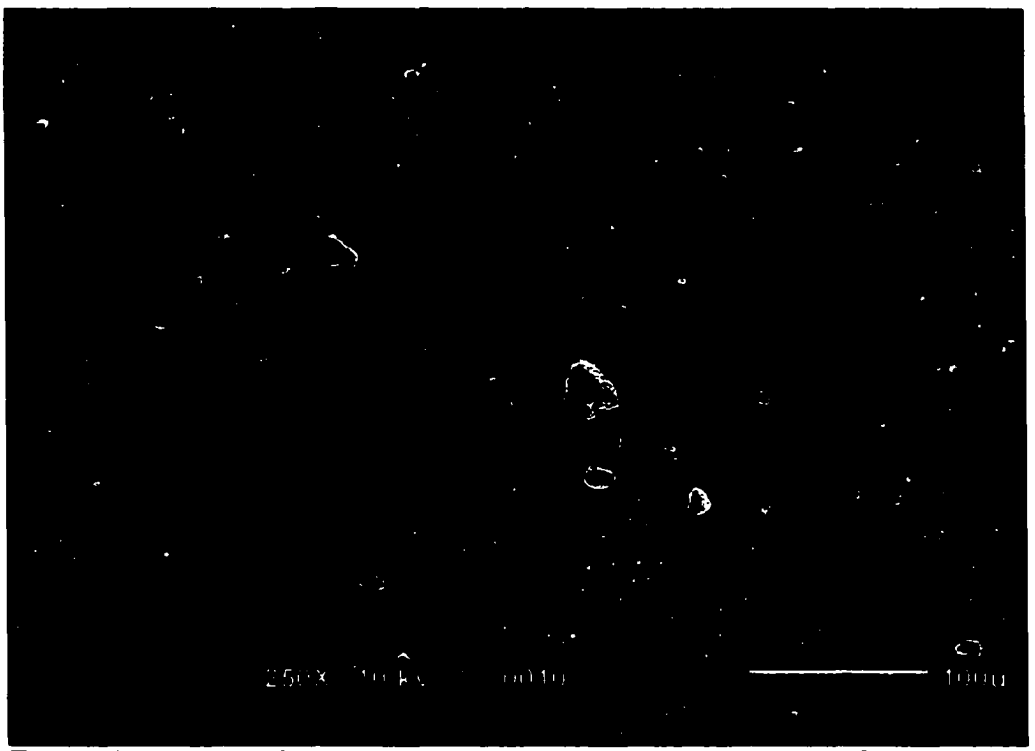

Fig. 4. Low magnification-scanning electron micrograph of the medium clay fraction from a Monona soil.

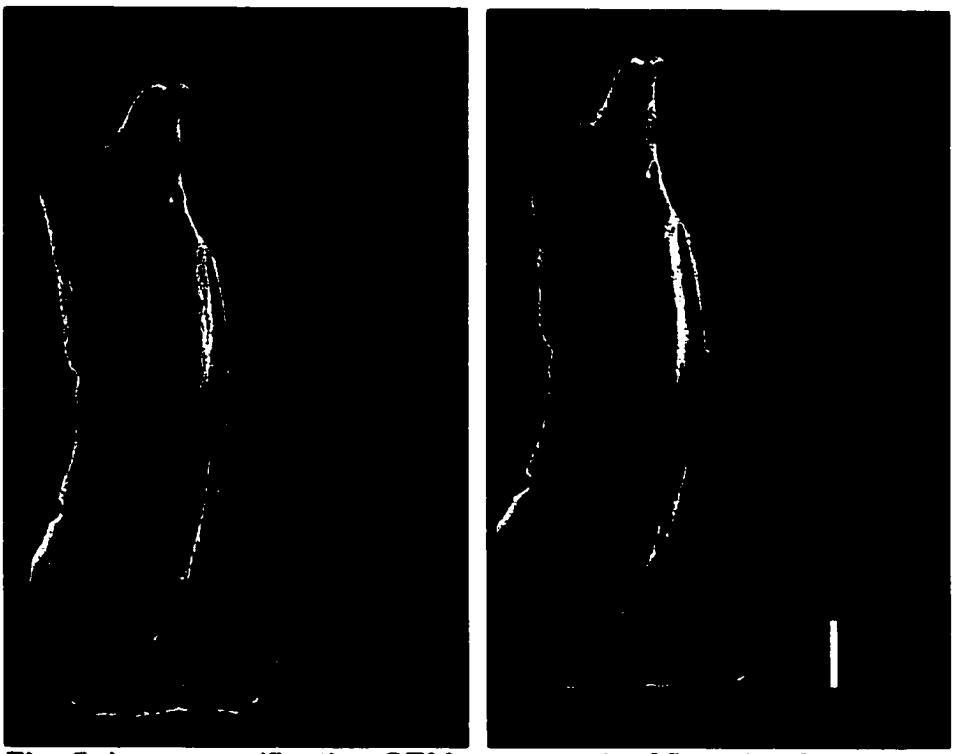

Fig. 5. Low magnification SEM stereo pair of fine clay fraction from a Monona soil. White bar represents 10 μm.

Fig. 6. Low magnification SEM stereo pair of fine clay fraction from a Monona soil. White bar represents 10 μm.

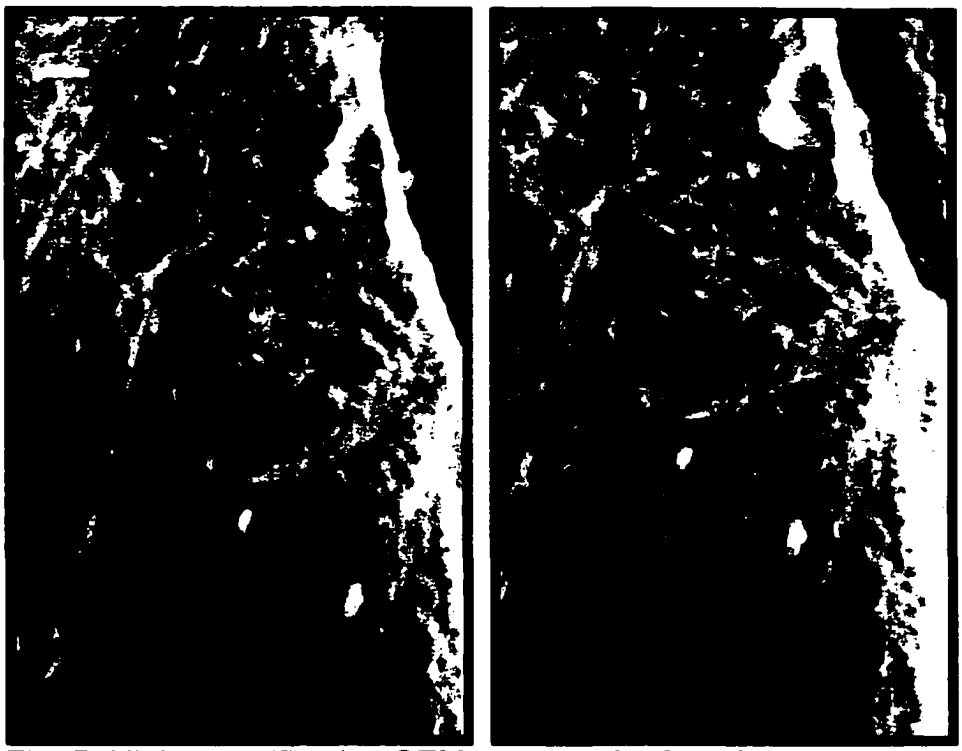

Fig. 7. High magnification SEM stereo pair of the fine clay fraction from a Monona soil. White bar represents 1 μm.

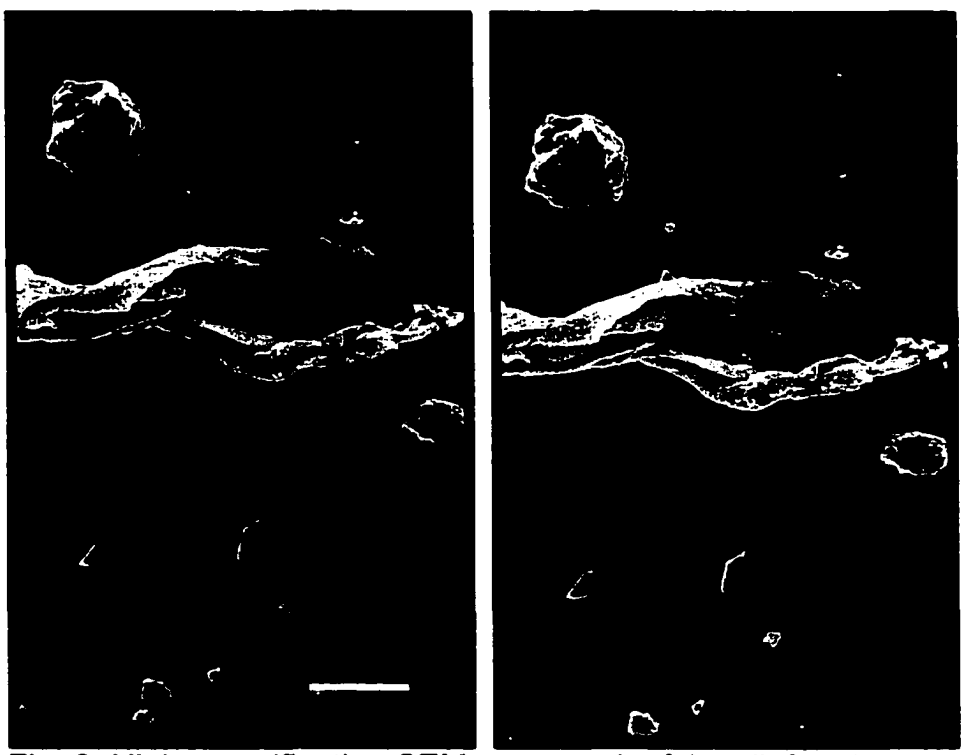

Fig. 8. High magnification SEM stereo pair of the silt fraction from a Monona soil. White bar represents 10 μm.

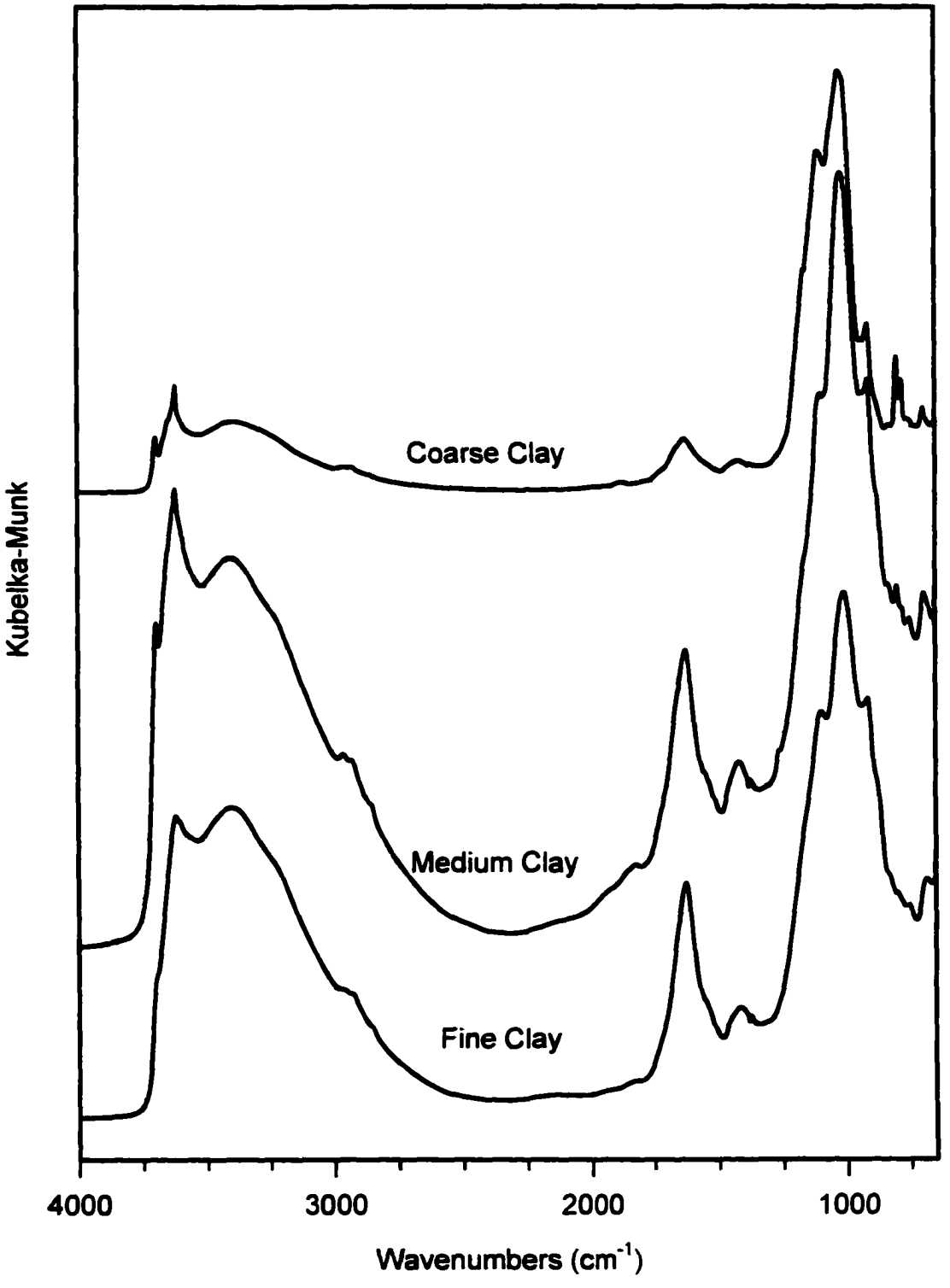


Fig. 9. FT-IR spectra of the clay fractions of the Monona soil (control).

Table 1. $^{14}C$ activity and $^{14}C$ specific activity in the Monona soil mineral fractions at Day-0 of the incubation.

| Sample | | $^{14}C$ Activity | | | $^{14}C$ Specific Activity | |
|---|---|---|---|---|---|---|
| | *weight (g)* | *dpm burn$^{-1}$ †* | *dpm g$^{-1}$* | *Bq g$^{-1}$* | *Bq mg$^{-1}$ C* | |
| Silt | 1.2234 | 220.1 | 179.9 | 2.60 | 584.5 | 526.5 |
| | 1.2413 | 188.2 | 151.6 | 2.13 | 478.7 | |
| | 1.2493 | 202.1 | 161.7 | 2.30 | 516.5 | |
| Coarse Clay | 0.5651 | 732.7 | 1297 | 21.2 | 574.1 | 565.8 |
| | 0.5539 | 704.9 | 1273 | 20.8 | 563.3 | |
| | 0.5709 | 722.5 | 1265 | 20.7 | 560.0 | |
| Medium Clay | 0.5495 | 743.4 | 1353 | 22.1 | 723.9 | 733.3 |
| | 0.5419 | 740.4 | 1366 | 22.4 | 731.2 | |
| | 0.5294 | 736.5 | 1391 | 22.8 | 744.7 | |
| Fine Clay | 0.2281 | 455.0 | 1995 | 32.8 | 836.9 | 847.2 |
| | 0.1660 | 339.2 | 2043 | 33.7 | 857.5 | |
| Control‡ | 1.2893 | 32.72 | 25.38 | 23.8 | | |
| | 1.3206 | 29.44 | 22.29 | | | |

† dpm: disintegration per minute
‡ silt + clay fraction without $^{14}C$ amendment

Table 2. $^{14}C$ activity and $^{14}C$ specific activity in the Monona soil mineral fractions at Day-360 of the incubation.

| Sample | | $^{14}C$ Activity | | | $^{14}C$ Specific Activity | |
|---|---|---|---|---|---|---|
| | *weight (g)* | *dpm burn$^{-1}$ †* | *dpm g$^{-1}$* | *Bq g$^{-1}$* | *Bq mg$^{-1}$ C* | |
| Silt | 1.0467 | 355.3 | 339.5 | 5.26 | 1182 | 1195 |
| | 1.0518 | 330.3 | 314.1 | 4.84 | 1087 | |
| | 1.1206 | 420.5 | 375.3 | 5.86 | 1316 | |
| Coarse Clay | 0.4561 | 728.4 | 1597 | 26.2 | 709.6 | 770.8 |
| | 0.4623 | 833.5 | 1803 | 29.7 | 802.5 | |
| | 0.4506 | 810.4 | 1798 | 29.6 | 800.5 | |
| Medium Clay | 0.5231 | 810.7 | 1550 | 25.4 | 831.1 | 855.5 |
| | 0.4556 | 724.1 | 1589 | 26.1 | 852.6 | |
| | 0.4799 | 789.2 | 1645 | 27.0 | 882.7 | |
| Fine Clay | 0.2083 | 735.8 | 3532 | 58.5 | 1490 | 1529 |
| | 0.1076 | 400.2 | 3719 | 61.6 | 1569 | |
| Control‡ | 1.2893 | 32.72 | 25.38 | 23.8 | | |
| | 1.3206 | 29.44 | 22.29 | | | |

† dpm: disintegration min$^{-1}$
‡ silt + clay fraction without $^{14}C$ amendment

Table 3. Organic C and total N content of the Monona soil mineral fractions.

| Fraction | % C | | | % N | | | C:N ratio |
|---|---|---|---|---|---|---|---|
| | | *average* | *std dev* | | *average* | *std dev* | |
| Silt | 0.45 | 0.45 | 0.007 | 0.05 | 0.05 | 0.007 | 9.9 |
| | 0.44 | | | 0.04 | | | |
| Coarse Clay | 3.70 | 3.70 | 0.007 | 0.33 | 0.34 | 0.007 | 11.0 |
| | 3.69 | | | 0.34 | | | |
| Medium Clay | 3.07 | 3.06 | 0.014 | 0.36 | 0.36 | 0.000 | 8.5 |
| | 3.05 | | | 0.36 | | | |
| Fine Clay | 3.93 | 3.93 | 0.007 | 0.41 | 0.41 | 0.000 | 9.6 |
| | 3.92 | | | 0.41 | | | |

# APPENDIX B

## RAW DATA FROM CHAPTER 3

(p. 153-165)

Table 1. Raw data of C for the Na-Clay + glucose systems.

| ----- Sample----- | | Added Glucose | | Added Glucose-C | | Glucose-C Recovered | | |
|---|---|---|---|---|---|---|---|---|
| Name | Weight | ----- µmol ----- | | ----- mg ----- | | mg | | |
| | --- g --- | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* | *$g^{-1}$ clay* | ------- % ------- | |
| | 0.2492 | 250 | 1003.2 | 18.00 | 72.23 | 69.29 | 95.9 | 98.3‡ |
| Na-SWa | 0.2528 | 250 | 988.9 | 18.00 | 71.20 | 71.62 | 100.6 | (3.3)§ |
| | nd† | nd | nd | nd | nd | nd | nd | |
| | 0.5048 | 500 | 990.5 | 36.00 | 71.32 | 56.62 | 79.4 | 81.2 |
| Na-Panther | 0.4928 | 500 | 1014.6 | 36.00 | 73.05 | 57.97 | 79.3 | (3.1) |
| | 0.5071 | 500 | 986.0 | 36.00 | 70.99 | 60.16 | 84.7 | |
| | 0.4975 | 500 | 1005.0 | 36.00 | 72.36 | 27.03 | 37.4 | 39.6 |
| Na-Saponite | 0.4811 | 500 | 1039.3 | 36.00 | 74.83 | 27.47 | 36.7 | (4.4) |
| | 0.4937 | 500 | 1012.8 | 36.00 | 72.92 | 32.60 | 44.7 | |
| | 0.5060 | 500 | 988.1 | 36.00 | 71.15 | 33.17 | 46.6 | 43.9 |
| Na-Otay | 0.4931 | 500 | 1014.0 | 36.00 | 73.01 | 29.73 | 40.7 | (3.0) |
| | 0.5104 | 500 | 979.6 | 36.00 | 70.53 | 31.39 | 44.5 | |
| Control | - | 250 | - | 18.00 | | 15.44 | 85.8 | 88.0 |
| (Glucose) | - | 250 | - | 18.00 | | 16.23 | 90.2 | (0.6) |

† not determined
‡ average
§ standard deviation in parenthesis

Table 2. Raw data of C for the Ca-Clay + glucose systems.

| ------ Sample------ | | Added Glucose | | Added Glucose-C | | Glucose-C Recovered | | |
|---|---|---|---|---|---|---|---|---|
| Name | Weight | ----- µmol ------ | | ----- mg ------ | | mg | | |
| | --- g --- | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* | *$g^{-1}$ clay* | ------ % ------ | |
| | 0.4999 | 500 | 1000.2 | 36.00 | 72.01 | 45.54 | 63.2 | 65.3† |
| Ca-SWa | 0.5050 | 500 | 990.1 | 36.00 | 71.29 | 48.92 | 68.6 | (2.9)‡ |
| | 0.5096 | 500 | 981.2 | 36.00 | 70.64 | 45.31 | 64.1 | |
| | 0.5107 | 500 | 979.0 | 36.00 | 70.49 | 38.83 | 55.1 | 56.9 |
| Ca-Panther | 0.5020 | 500 | 996.0 | 36.00 | 71.71 | 42.83 | 59.7 | (2.5) |
| | 0.5009 | 500 | 998.2 | 36.00 | 71.87 | 40.09 | 55.8 | |
| | 0.4905 | 500 | 1019.4 | 36.00 | 73.39 | 33.81 | 46.1 | 43.9 |
| Ca-Saponite | 0.4986 | 500 | 1002.8 | 36.00 | 72.20 | 30.12 | 41.7 | (2.2) |
| | 0.5015 | 500 | 997.0 | 36.00 | 71.78 | 31.43 | 43.8 | |
| | 0.5119 | 500 | 976.8 | 36.00 | 70.33 | 36.23 | 51.5 | 50.7 |
| Ca-Otay | 0.4924 | 500 | 1015.4 | 36.00 | 73.11 | 34.49 | 47.2 | (3.2) |
| | 0.5144 | 500 | 972.0 | 36.00 | 69.98 | 37.71 | 53.5 | |

† average
‡ standard deviation in parenthesis

Table 3. Raw data of C for the Cu-Clay + glucose systems.

| ------ Sample----- | | Added Glucose | | Added Glucose-C | | Glucose-C Recovered | | |
|---|---|---|---|---|---|---|---|---|
| Name | Weight | ----- µmol ------ | | ----- mg ------ | | mg | | |
| | *--- g ---* | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* | *$g^{-1}$ clay* | ------ % ------ | |
| | 0.4891 | 500 | 1022.3 | 36.00 | 73.60 | 15.75 | 21.4 | 22.7‡ |
| Cu-SWa | 0.4938 | 500 | 1012.6 | 36.00 | 72.90 | 17.46 | 23.9 | (1.8)§ |
| | 0.5059 | 500 | 988.3 | 36.00 | 71.16 | nd† | nd | |
| | 0.5139 | 500 | 973.0 | 36.00 | 70.05 | 0.79 | 1.1 | 2.5 |
| Cu-Panther | 0.5112 | 500 | 978.1 | 36.00 | 70.42 | 3.41 | 4.8 | (2.0) |
| | 0.4978 | 500 | 1004.4 | 36.00 | 72.32 | 1.12 | 1.6 | |
| | 0.5181 | 500 | 965.1 | 36.00 | 69.48 | 13.09 | 18.8 | 10.4 |
| Cu-Saponite | 0.5081 | 500 | 984.1 | 36.00 | 70.85 | 1.40 | 2.0 | (11.9) |
| | 0.5013 | 500 | 997.4 | 36.00 | 71.81 | nd | nd | |
| | 0.4957 | 500 | 1008.7 | 36.00 | 72.62 | 5.20 | 7.2 | 4.0 |
| Cu-Otay | 0.5057 | 500 | 988.7 | 36.00 | 71.19 | 0.55 | 0.8 | (4.5) |
| | 0.4946 | 500 | 1010.9 | 36.00 | 72.79 | nd | nd | |

† not determined
‡ average
§ standard deviation in parenthesis

Table 4. Raw data of C for the Al-Clay + glucose systems.

| ----- Sample----- | | Added Glucose | | Added Glucose-C | | Glucose-C Recovered | | |
|---|---|---|---|---|---|---|---|---|
| Name | Weight | ----- µmol ------ | | ----- mg ------ | | mg | | |
| | --- g --- | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* | *$g^{-1}$ clay* | ------- % ------- | |
| | 0.2453 | 250 | 1019.2 | 18.00 | 73.38 | 59.76 | 81.4 | 83.9‡ |
| Al-SWa | 0.2570 | 250 | 972.8 | 18.00 | 70.04 | 60.51 | 86.4 | (3.5)§ |
| | lost | -- | -- | -- | -- | -- | -- | |
| | 0.5073 | 500 | 985.6 | 36.00 | 70.96 | 40.33 | 56.8 | 59.3 |
| Al-Panther | 0.4991 | 500 | 1001.8 | 36.00 | 72.13 | 43.11 | 59.8 | (2.2) |
| | 0.4920 | 500 | 1016.3 | 36.00 | 73.17 | 44.77 | 61.2 | |
| | 0.5012 | 500 | 997.6 | 36.00 | 71.83 | nd† | nd | 29.7 |
| Al-Saponite | 0.4962 | 500 | 1007.7 | 36.00 | 72.55 | 22.02 | 30.4 | (1.0) |
| | 0.5032 | 500 | 993.6 | 36.00 | 71.54 | 20.74 | 29.0 | |
| | 0.5107 | 500 | 979.0 | 36.00 | 70.49 | 10.62 | 15.1 | 18.3 |
| Al-Otay | 0.4861 | 500 | 1028.6 | 36.00 | 74.06 | 12.96 | 17.5 | (3.7) |
| | 0.5038 | 500 | 992.5 | 36.00 | 71.46 | 16.02 | 22.4 | |

† not determined
‡ average
§ standard deviation in parenthesis

Table 5. Raw data of C for the Fe-Clay + glucose systems.

| ------ Sample----- | | Added Glucose | | Added Glucose-C | | Glucose-C Recovered | | |
|---|---|---|---|---|---|---|---|---|
| Name | Weight | ----- µmol ------ | | ----- mg ------ | | mg | | |
| | *— g —* | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* | *$g^{-1}$ clay* | ------ % ------ | |
| | 0.2573 | 250 | 971.6 | 18.00 | 69.96 | 56.47 | 80.7 | 82.0† |
| Fe-SWa | 0.2444 | 250 | 1022.9 | 18.00 | 73.65 | 61.06 | 82.9 | (1.1)‡ |
| | 0.2519 | 250 | 992.5 | 18.00 | 71.46 | 58.83 | 82.3 | |
| | 0.2438 | 250 | 1025.4 | 18.00 | 73.83 | 66.89 | 90.6 | 91.9 |
| Fe-Panther | 0.2487 | 250 | 1005.2 | 18.00 | 72.38 | 70.53 | 97.5 | (5.0) |
| | 0.2444 | 250 | 1022.9 | 18.00 | 73.65 | 64.54 | 87.6 | |
| | 0.2557 | 250 | 977.7 | 18.00 | 70.39 | 64.58 | 91.7 | 83.9 |
| Fe-Saponite | 0.2593 | 250 | 964.1 | 18.00 | 69.42 | 62.90 | 90.6 | (12.5) |
| | 0.2444 | 250 | 1022.9 | 18.00 | 73.65 | 51.18 | 69.5 | |
| | 0.2440 | 250 | 1024.6 | 18.00 | 73.77 | 61.36 | 83.2 | 78.2 |
| Fe-Otay | 0.2244 | 250 | 1114.1 | 18.00 | 80.21 | 61.12 | 76.2 | (4.4) |
| | 0.2225 | 250 | 1123.6 | 18.00 | 80.90 | 60.79 | 75.1 | |

† average
‡ standard deviation in parenthesis

Table 6. Raw data of C for the goehtite + glucose systems.

| Sample | | Added Glucose | | Added Glucose-C | | Glucose-C Recovered | | |
|---|---|---|---|---|---|---|---|---|
| Name | Weight | µmol | | mg | | mg | | |
| | *g* | *Total* | *g⁻¹-goethite* | *Total* | *g⁻¹-goethite* | | % | |
| | 0.5009 | 500 | 998.2 | 36.00 | 71.87 | 40.14 | 55.86 | 58.3† |
| Goe-00 | 0.4983 | 500 | 1003.4 | 36.00 | 72.25 | 36.45 | 50.46 | (9.3)‡ |
| | 0.5107 | 500 | 979.0 | 36.00 | 70.49 | 48.34 | 68.57 | |
| | 0.4951 | 500 | 1009.9 | 36.00 | 72.71 | 54.91 | 75.52 | 80.1 |
| Goe-20 | 0.4894 | 500 | 1021.7 | 36.00 | 73.56 | 57.54 | 78.22 | (5.7) |
| | 0.4842 | 500 | 1032.6 | 36.00 | 74.35 | 64.35 | 86.55 | |
| | 0.4731 | 500 | 1056.9 | 36.00 | 76.09 | 66.40 | 87.27 | 83.2 |
| Goe-52 | 0.4880 | 500 | 1024.6 | 36.00 | 73.77 | 60.76 | 82.36 | (3.7) |
| | 0.4823 | 500 | 1036.7 | 36.00 | 74.64 | 59.77 | 80.08 | |
| | 0.4934 | 500 | 1013.4 | 36.00 | 72.96 | 54.79 | 75.10 | 79.7 |
| Goe-94 | 0.4762 | 500 | 1050.0 | 36.00 | 75.60 | 63.02 | 83.36 | (4.2) |
| | 0.4883 | 500 | 1024.0 | 36.00 | 73.73 | 59.48 | 80.67 | |

† average
‡ standard deviation in parenthesis

Table 7. Soluble organic C in clay + glucose systems.†

| Clay System | weight | C found Solution | | | |
|---|---|---|---|---|---|
| | | Total | $g^{-1}$ clay | | |
| | *g* | *mg* | | % | |
| Na-SWa | 0.2492 | 19.07 | 76.52 | 105.9 | 104.0 |
| | 0.2528 | 18.38 | 72.72 | 102.1 | (2.7)† |
| Na-Panther | 0.5048 | 36.87 | 73.05 | 102.4 | 101.4 |
| | 0.4928 | 36.71 | 74.49 | 102.0 | (1.5) |
| | 0.5071 | 35.88 | 70.76 | 99.7 | |
| Na-Saponite | 0.4975 | 38.06 | 76.50 | 105.7 | 101.4 |
| | 0.4811 | 37.59 | 78.13 | 104.4 | (6.5) |
| | 0.4937 | 33.82 | 68.50 | 93.9 | |
| Na-Otay | 0.5060 | 37.15 | 73.42 | 103.2 | 102.9 |
| | 0.4931 | 36.90 | 74.83 | 102.5 | (0.3) |
| | 0.5104 | 37.04 | 72.57 | 102.9 | |
| Ca-SWa | 0.4999 | 37.09 | 74.20 | 103.0 | 102.7 |
| | 0.5050 | 37.07 | 73.40 | 103.0 | (0.6) |
| | 0.5050 | 36.71 | 72.69 | 102.0 | |
| Ca-Panther | 0.5107 | 36.65 | 71.77 | 101.8 | 102.0 |
| | 0.5020 | 37.01 | 73.73 | 102.8 | (0.7) |
| | 0.5009 | 36.52 | 72.90 | 101.4 | |
| Ca-Saponite | 0.4905 | 36.90 | 75.23 | 102.5 | 102.8 |
| | 0.4986 | 37.07 | 74.34 | 103.0 | (0.2) |
| | 0.5015 | 37.01 | 73.80 | 102.8 | |
| Ca-Otay | 0.5119 | 37.62 | 73.48 | 104.5 | 105.3 |
| | 0.4924 | 37.40 | 75.95 | 103.9 | (1.9) |
| | 0.5144 | 38.69 | 75.21 | 107.5 | |
| Cu-SWa | 0.4891 | 34.01 | 69.54 | 94.5 | 95.3 |
| | 0.4938 | 34.12 | 69.10 | 94.8 | (1.2) |
| | 0.4938 | 34.84 | 70.55 | 96.8 | |
| Cu-Panther | 0.5139 | 35.77 | 69.61 | 99.4 | 101.8 |
| | 0.5112 | 37.15 | 72.67 | 103.2 | (2.1) |
| | 0.4978 | 36.98 | 74.29 | 102.7 | |

† soluble organic C determined by the $K_2Cr_2O_7$ method.

Table 7. Soluble organic C in clay + glucose systems. *(continuation)*

| Clay System | weight | C found Solution | | | |
|---|---|---|---|---|---|
| | | Total | $g^{-1}$ clay | | |
| | *g* | *mg* | | % | |
| Cu-Saponite | 0.5181 | 34.67 | 66.92 | 96.3 | 95.5 |
| | 0.5081 | 34.07 | 67.05 | 94.6 | (0.8) |
| | 0.5013 | 34.40 | 68.62 | 95.5 | |
| Cu-Otay | 0.4957 | 37.09 | 74.83 | 103.0 | 102.4 |
| | 0.5057 | 36.65 | 72.48 | 101.8 | (0.6) |
| | 0.4946 | 36.82 | 74.44 | 102.3 | |
| Al-SWa | 0.2453 | 20.08 | 81.84 | 111.5 | 109.6 |
| | 0.2570 | 19.39 | 75.45 | 107.7 | (2.7) |
| Al-Panther | 0.5073 | 34.56 | 68.13 | 96.0 | 95.0 |
| | 0.4991 | 34.12 | 68.37 | 94.8 | (0.9) |
| | 0.4920 | 33.90 | 68.91 | 94.2 | |
| Al-Saponite | 0.5012 | 34.89 | 69.62 | 96.9 | 99.0 |
| | 0.4962 | 35.83 | 72.20 | 99.5 | (1.9) |
| | 0.5032 | 36.21 | 71.97 | 100.6 | |
| Al-Otay | 0.5107 | 36.43 | 71.34 | 101.2 | 101.4 |
| | 0.4861 | 36.82 | 75.74 | 102.3 | (0.8) |
| | 0.5038 | 36.27 | 71.99 | 100.7 | |
| Fe-SWa | 0.2573 | 19.59 | 76.13 | 108.8 | 108.2 |
| | 0.2444 | 19.32 | 79.04 | 107.3 | (0.8) |
| | 0.2444 | 19.50 | 79.80 | 108.4 | |
| Fe-Panther | 0.2438 | 19.50 | 80.00 | 108.4 | 108.2 |
| | 0.2487 | 19.82 | 79.68 | 110.1 | (1.9) |
| | 0.2444 | 19.13 | 78.27 | 106.3 | |
| Fe-Saponite | 0.2557 | 18.76 | 73.35 | 104.2 | 105.6 |
| | 0.2593 | 19.15 | 73.86 | 106.4 | (1.2) |
| | 0.2444 | 19.11 | 78.19 | 106.2 | |
| Fe-Otay | 0.2440 | 19.25 | 78.91 | 107.0 | 109.3 |
| | 0.2244 | 19.80 | 88.21 | 110.0 | (2.1) |
| | 0.2225 | 19.96 | 89.72 | 110.9 | |

† Standard deviation in parenthesis.

Table 8. Soluble organic C in goethite + glucose systems.[†]

| Goethite | weight | C found Solution | | | |
|---|---|---|---|---|---|
| | | Total | $g^{-1}$ goethite | | |
| | *g* | *mg* | | % | |
| | 0.5009 | 40.10 | 80.06 | 111.4 | 108.2 |
| Goe-00 | 0.4983 | 38.60 | 77.47 | 107.2 | 2.8 |
| | 0.5107 | 38.18 | 74.75 | 106.0 | |
| | 0.4951 | 37.53 | 75.81 | 104.3 | 106.0 |
| Goe-20 | 0.4894 | 38.44 | 78.55 | 106.8 | 1.5 |
| | 0.4842 | 38.50 | 79.51 | 106.9 | |
| | 0.4731 | 38.60 | 81.60 | 107.2 | 106.1 |
| Goe-52 | 0.4880 | 38.12 | 78.12 | 105.9 | 1.1 |
| | 0.4823 | 37.86 | 78.49 | 105.2 | |
| | 0.4934 | 40.26 | 81.60 | 111.8 | 110.1 |
| Goe-94 | 0.4762 | 39.73 | 83.43 | 110.4 | 1.9 |
| | 0.4883 | 38.93 | 79.72 | 108.1 | |

Table 9. Evolved $CO_2$-C in clay + glucose systems.

| Clay System | weight | Added C | Evolved $CO_2$-C | | |
|---|---|---|---|---|---|
| | | | $g^{-1}$ clay | | |
| | *g* | *mg* | | % | |
| Na-SWa | 0.2492 | 72.23 | 0.05 | 0.06 | 0.1 |
| | 0.2528 | 71.20 | 0.05 | 0.08 | (0.0) |
| Na-Panther | 0.5048 | 71.32 | 0.06 | 0.09 | 0.1 |
| | 0.4928 | 73.05 | 0.05 | 0.06 | (0.0) |
| | 0.5071 | 70.99 | 0.04 | 0.06 | |
| Na-Saponite | 0.4975 | 72.36 | 0.08 | 0.11 | 0.1 |
| | 0.4811 | 74.83 | 0.08 | 0.11 | (0.0) |
| | 0.4937 | 72.92 | 0.02 | 0.03 | |
| Na-Otay | 0.5060 | 71.15 | 0.00 | 0.00 | 0.0 |
| | 0.4931 | 73.01 | 0.00 | 0.00 | (0.0) |
| | 0.5104 | 70.53 | 0.00 | 0.00 | |
| Ca-SWa | 0.4999 | 72.01 | 0.02 | 0.03 | 0.0 |
| | 0.0505 | 713.30 | 0.20 | 0.03 | (0.0) |
| | 0.5096 | 70.64 | 0.02 | 0.03 | |
| Ca-Panther | 0.5107 | 70.49 | 0.02 | 0.02 | 0.0 |
| | 0.5020 | 71.71 | 0.02 | 0.03 | (0.0) |
| | 0.5009 | 71.87 | 0.02 | 0.03 | |
| Ca-Saponite | 0.4905 | 73.39 | 0.00 | 0.00 | 0.0 |
| | 0.4986 | 72.20 | 0.00 | 0.01 | (0.0) |
| | 0.5015 | 71.78 | 0.00 | 0.00 | |
| Ca-Otay | 0.5119 | 70.33 | 0.00 | 0.00 | 0.0 |
| | 0.4924 | 73.11 | 0.00 | 0.00 | (0.0) |
| | 0.5144 | 69.98 | 0.00 | 0.01 | |
| Cu-SWa | 0.4891 | 73.60 | 0.00 | 0.00 | 0.0 |
| | 0.4938 | 72.90 | 0.00 | 0.00 | (0.0) |
| | 0.5059 | 71.16 | 0.00 | 0.00 | |
| Cu-Panther | 0.5139 | 70.05 | 0.00 | 0.00 | 0.0 |
| | 0.5112 | 70.42 | 0.00 | 0.00 | (0.0) |
| | 0.4978 | 72.32 | 0.00 | 0.00 | |

Table 9. Evolved $CO_2$-C in clay + glucose systems. *(continuation)*

| Clay System | weight | Added C | Evolved $CO_2$-C | | |
|---|---|---|---|---|---|
| | | | $g^{-1}$ clay | | |
| | *g* | *mg* | | % | |
| Cu-Saponite | 0.5181 | 69.48 | 0.03 | 0.04 | 0.0 |
| | 0.5081 | 70.85 | 0.03 | 0.04 | (0.0) |
| | 0.5013 | 71.81 | 0.02 | 0.03 | |
| Cu-Otay | 0.4957 | 72.62 | 0.00 | 0.00 | 0.0 |
| | 0.5057 | 71.19 | 0.00 | 0.00 | (0.0) |
| | 0.4946 | 72.79 | 0.00 | 0.00 | |
| Al-SWa | 0.2453 | 73.38 | 0.01 | 0.02 | 0.0 |
| | 0.2570 | 70.04 | 0.01 | 0.02 | (0.0) |
| Al-Panther | 0.5073 | 70.96 | 0.00 | 0.00 | 0.0 |
| | 0.4991 | 72.13 | 0.00 | 0.00 | (0.0) |
| | 0.4920 | 73.17 | 0.01 | 0.02 | |
| Al-Saponite | 0.5012 | 71.83 | 0.00 | 0.00 | 0.0 |
| | 0.4962 | 72.55 | 0.00 | 0.00 | (0.0) |
| | 0.5032 | 71.54 | 0.00 | 0.00 | |
| Al-Otay | 0.5107 | 70.49 | 0.00 | 0.00 | 0.0 |
| | 0.4861 | 74.06 | 0.00 | 0.00 | (0.0) |
| | 0.5038 | 71.46 | 0.00 | 0.00 | |
| Fe-SWa | 0.2573 | 69.96 | 0.05 | 0.07 | 0.1 |
| | 0.2444 | 73.65 | 0.04 | 0.06 | (0.0) |
| | 0.2519 | 71.46 | 0.05 | 0.07 | |
| Fe-Panther | 0.2438 | 73.83 | 0.03 | 0.04 | 0.1 |
| | 0.2487 | 72.38 | 0.04 | 0.05 | (0.2) |
| | 0.2444 | 73.65 | 0.24 | 0.32 | |
| Fe-Saponite | 0.2557 | 70.39 | 0.22 | 0.31 | 0.2 |
| | 0.2593 | 69.42 | 0.15 | 0.21 | (0.1) |
| | 0.2444 | 73.65 | 0.11 | 0.15 | |
| Fe-Otay | 0.2440 | 73.77 | 0.04 | 0.06 | 0.0 |
| | 0.2244 | 80.21 | 0.03 | 0.04 | (0.0) |
| | 0.2225 | 80.90 | 0.03 | 0.04 | |

Standard deviation in parenthesis.

Table 10. Evolved $CO_2$-C in goethite + glucose systems.

| Goethite | weight | Added C | Evolved $CO_2$-C | | |
|---|---|---|---|---|---|
| | | | $g^{-1}$ goethite | | |
| | *g* | *mg* | | % | |
| Goe-00 | 0.5009 | 71.87 | 0.02 | 0.02 | 0.0 |
| | 0.4983 | 72.25 | 0.03 | 0.05 | (0.0)† |
| | 0.5107 | 70.49 | 0.02 | 0.02 | |
| Goe-20 | 0.4951 | 72.71 | 0.02 | 0.02 | 0.0 |
| | 0.4894 | 73.56 | 0.02 | 0.02 | (0.0) |
| | 0.4842 | 74.35 | 0.02 | 0.03 | |
| Goe-52 | 0.4731 | 76.09 | 0.02 | 0.02 | 0.0 |
| | 0.4880 | 73.77 | 0.02 | 0.03 | (0.0) |
| | 0.4823 | 74.64 | 0.02 | 0.03 | |
| Goe-94 | 0.4934 | 72.96 | 0.03 | 0.04 | 0.0 |
| | 0.4762 | 75.60 | 0.03 | 0.03 | (0.0) |
| | 0.4883 | 73.73 | 0.03 | 0.04 | |

† Standard deviation in parenthesis.

# APPENDIX C

## RAW DATA FROM CHAPTER 4

(p. 166-193)

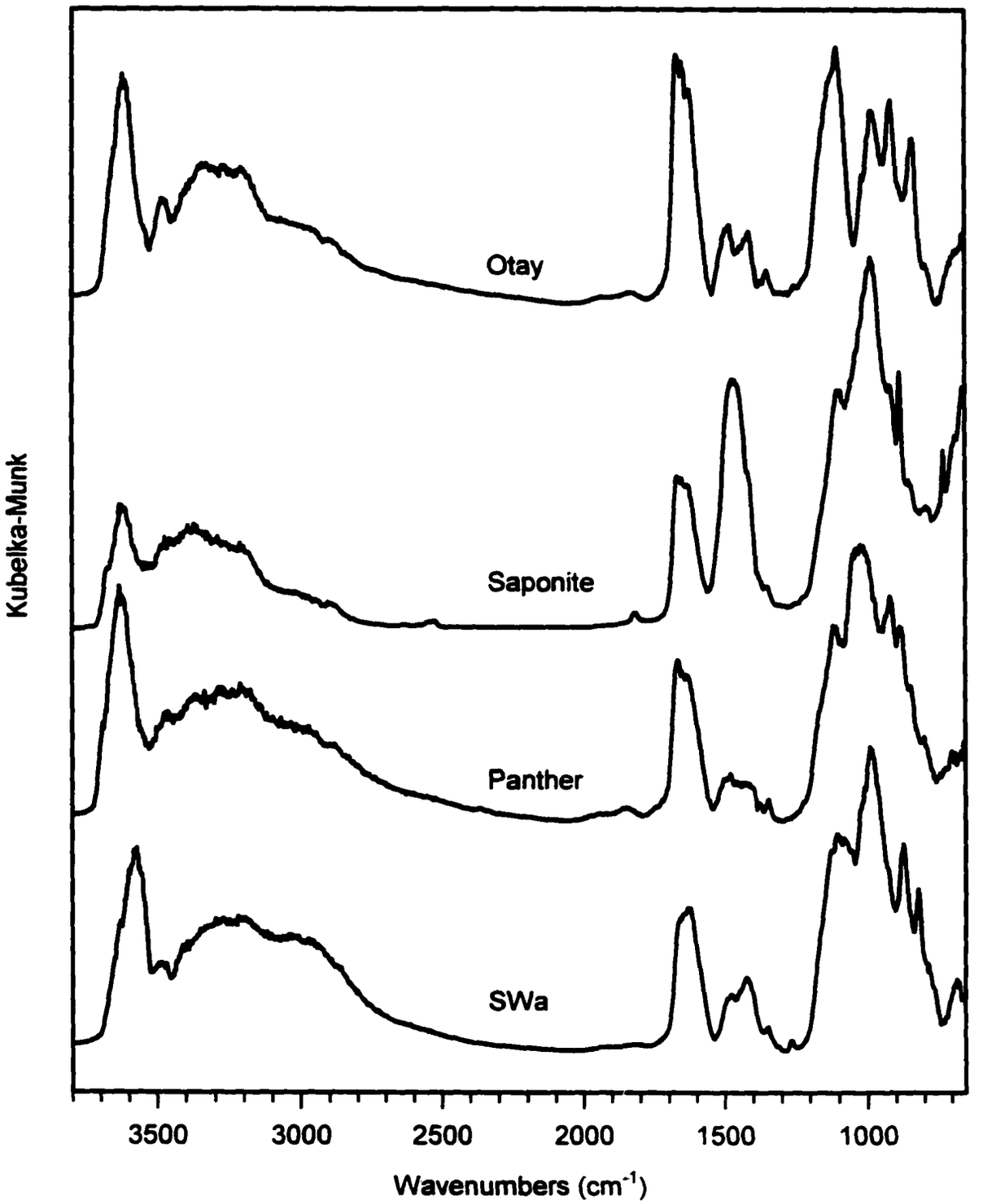


Fig. 1. FT-IR spectra of Na-clay + arginine + glucose systems.

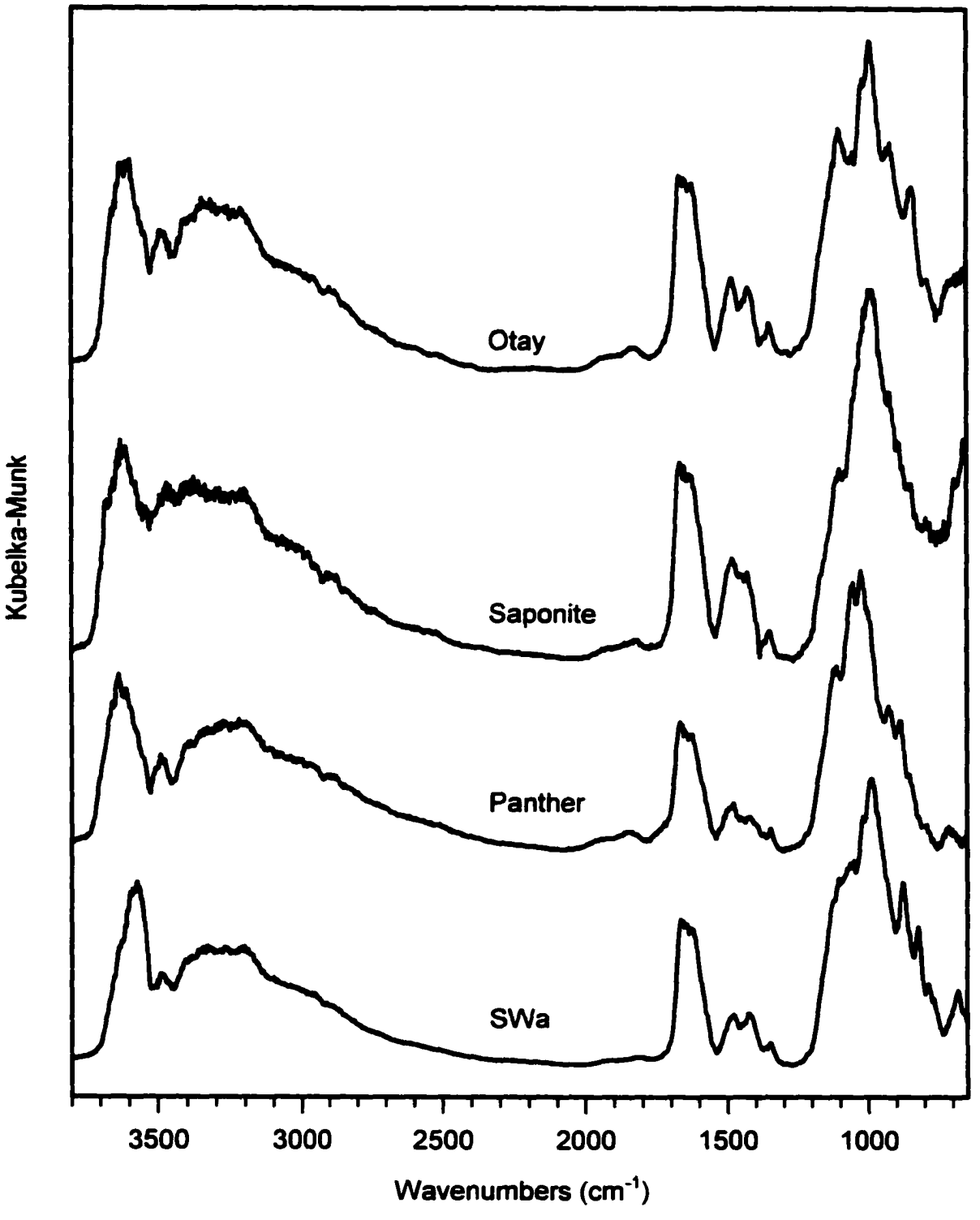


Fig. 2. FT-IR spectra of Ca-clay + arginine + glucose systems.

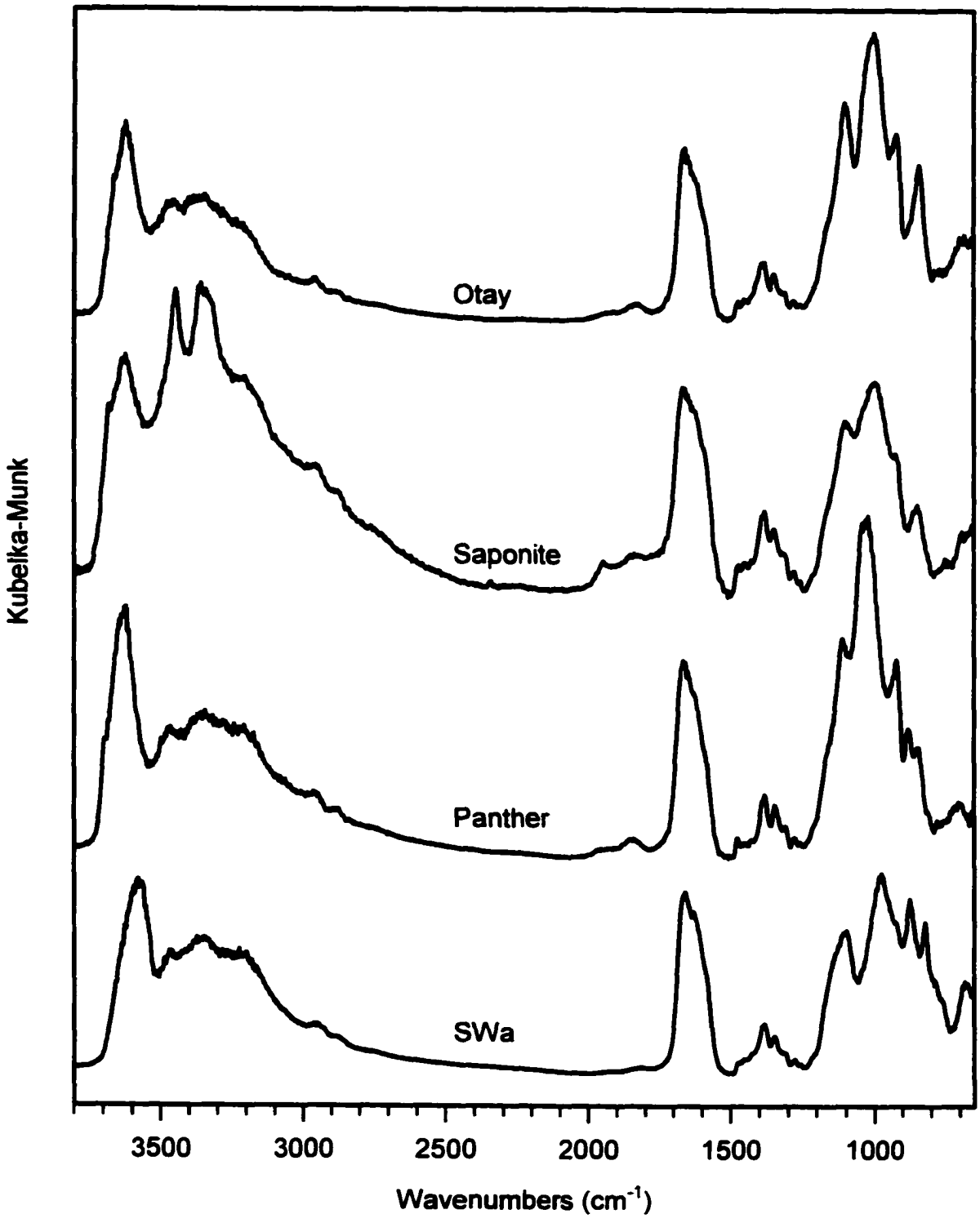


Fig. 3. FT-IR spectra of Cu-clay + arginine + glucose systems.

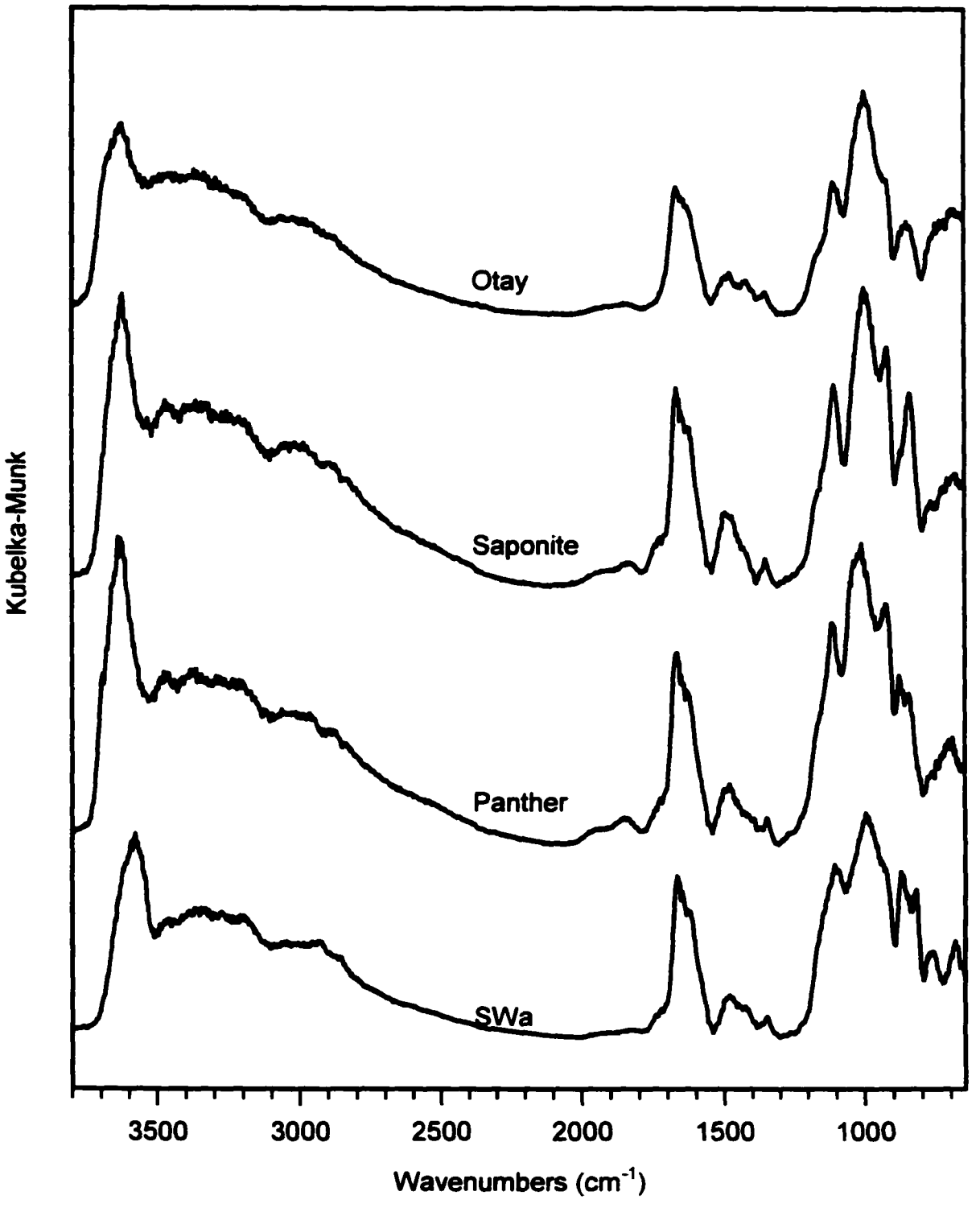


Fig. 4. FT-IR spectra of Al-clay + arginine + glucose systems.

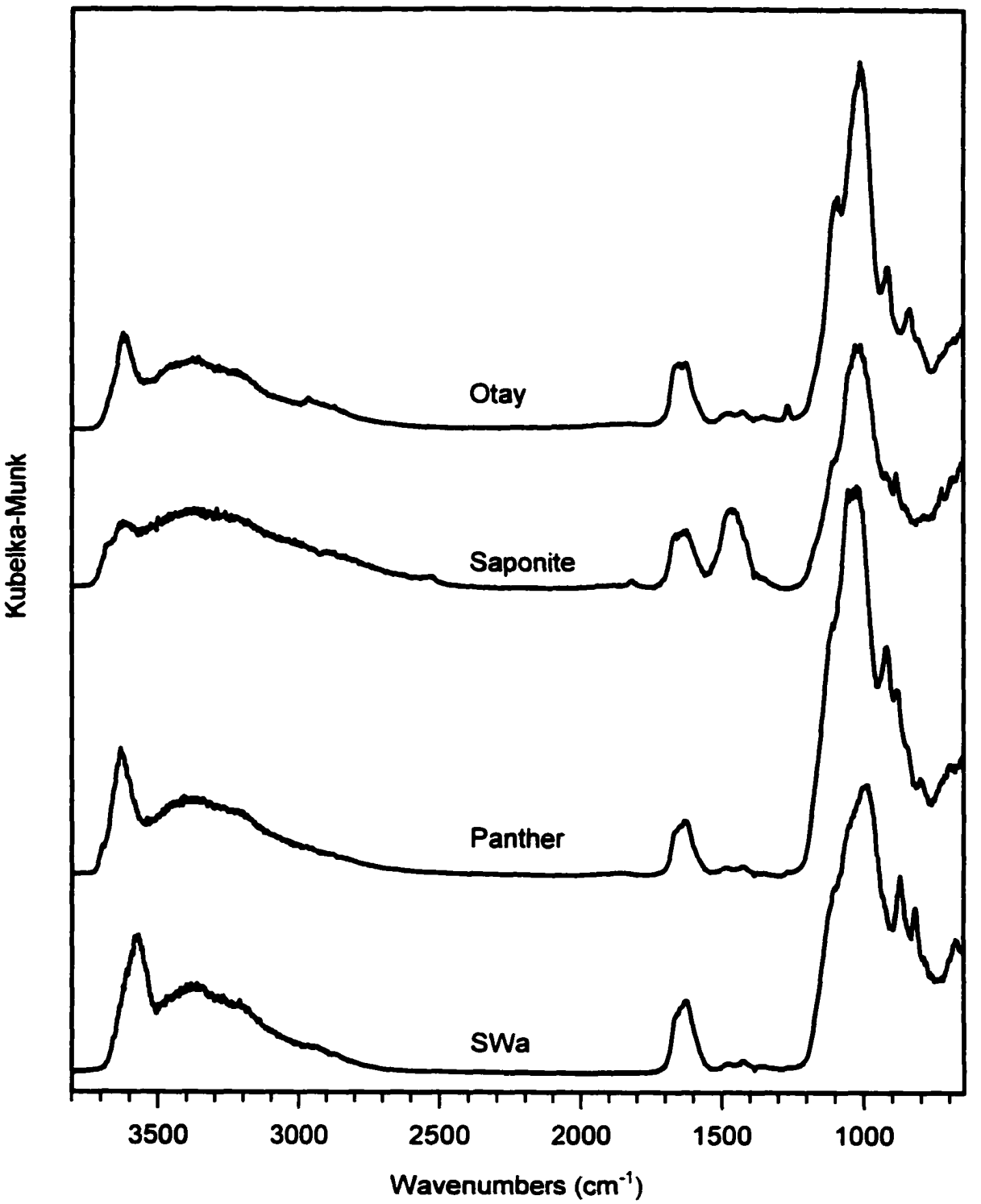


Fig. 5. FT-IR spectra of Fe-clay + arginine + glucose systems.

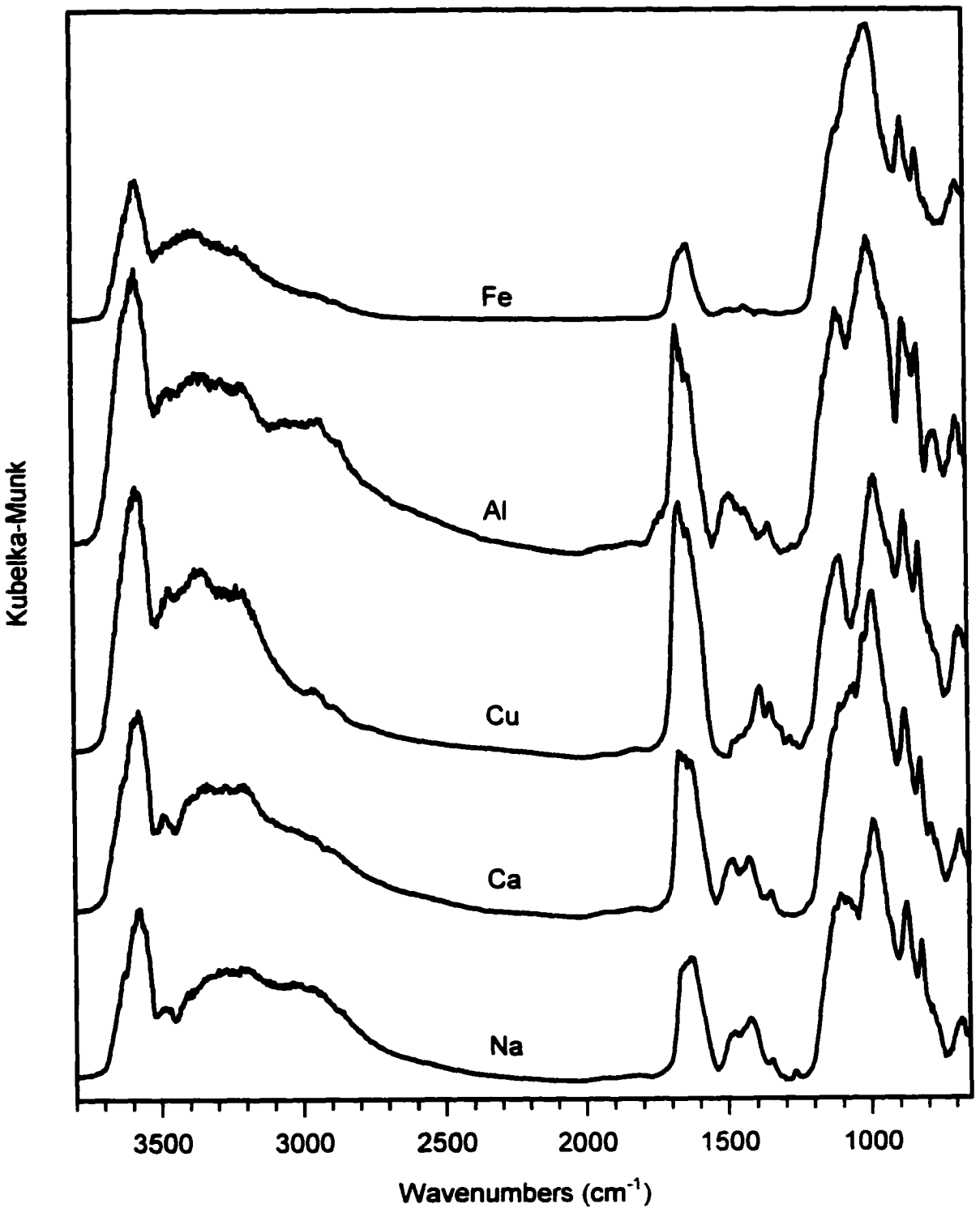


Fig. 6. FT-IR spectra of $M^{n+}$-SWa + arginine + glucose systems.

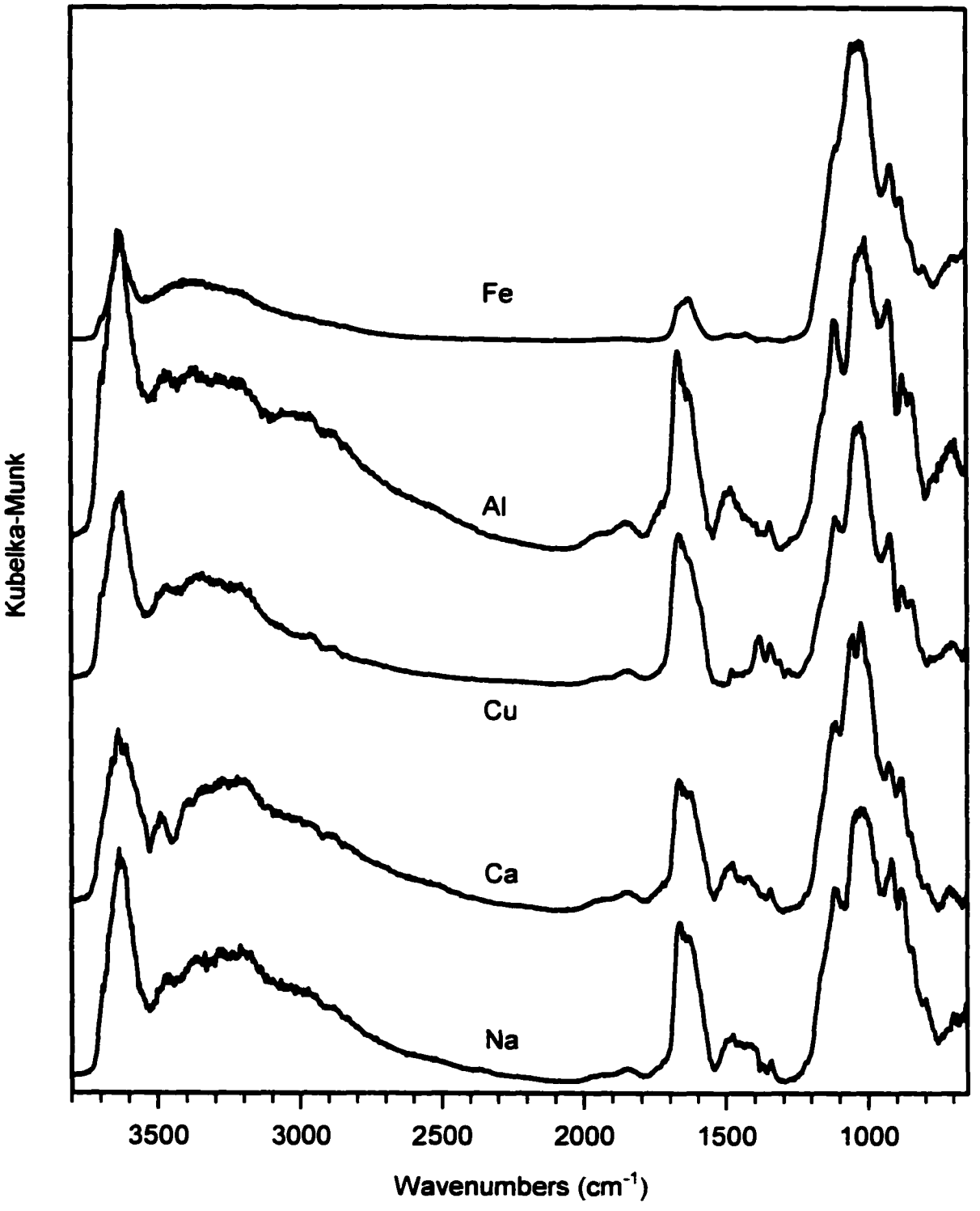


Fig. 7. FT-IR spectra of $M^{n+}$-Panther + arginine + glucose systems.

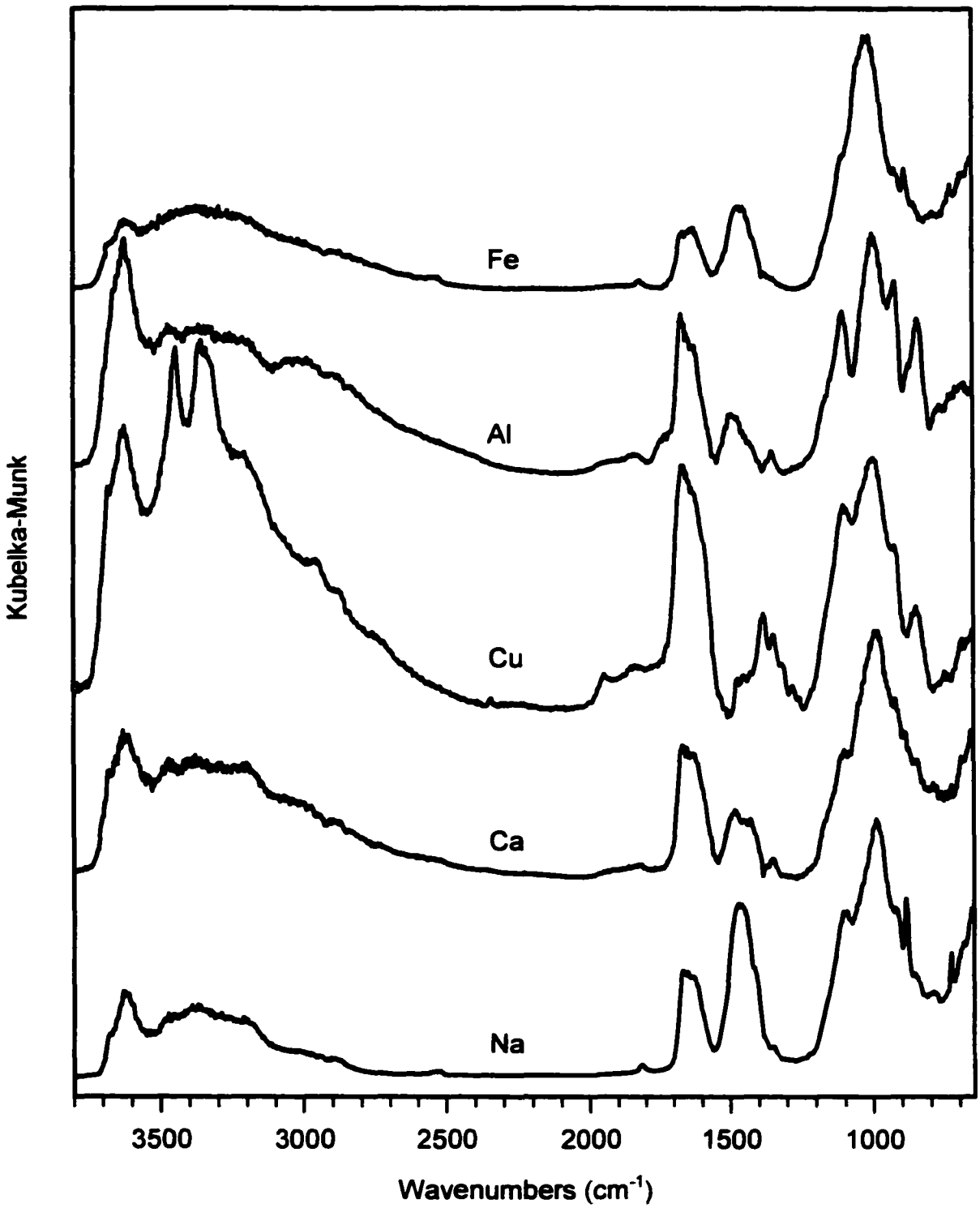


Fig. 8. FT-IR spectra of $M^{n+}$-Saponite + arginine + glucose systems.

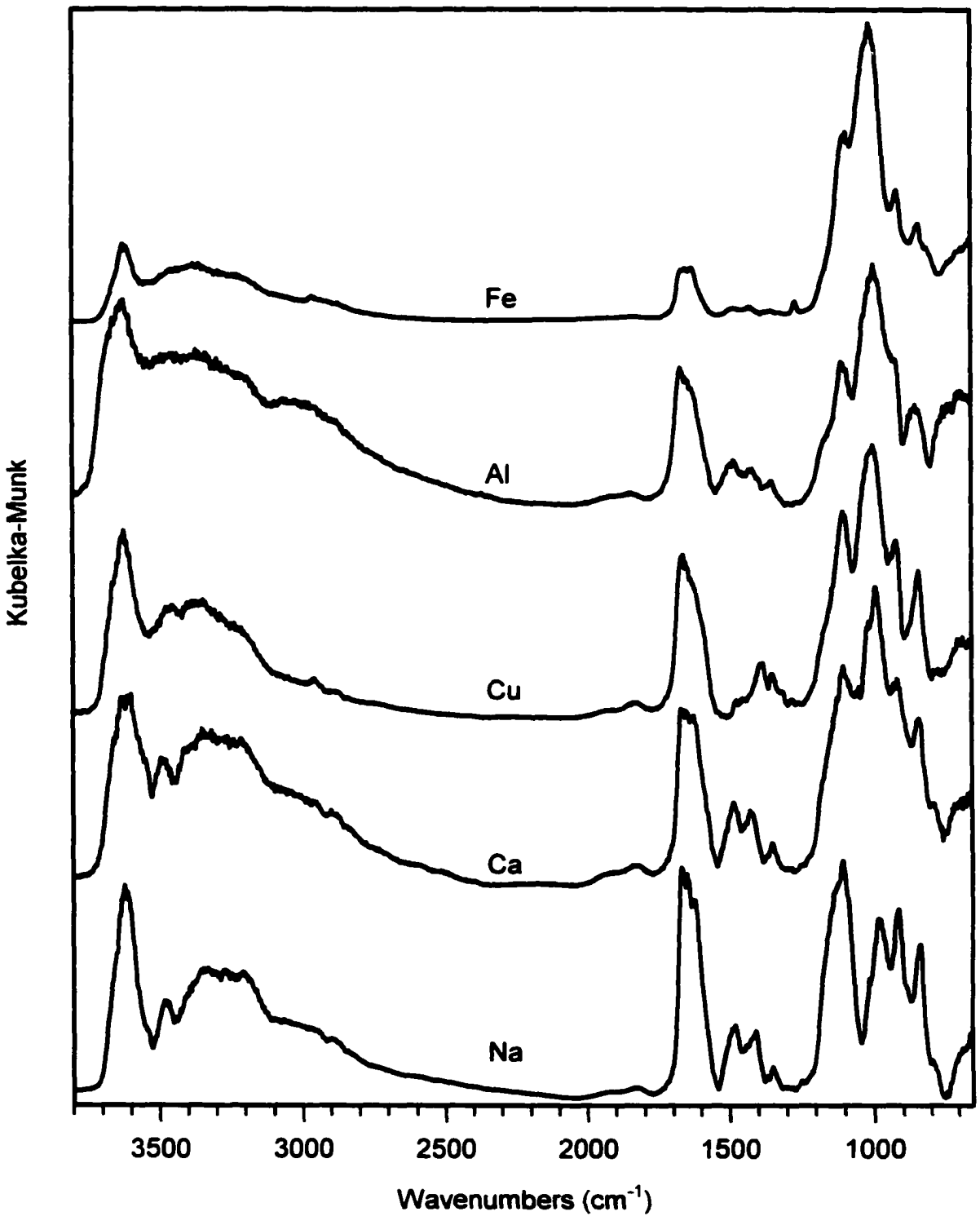


Fig. 9. FT-IR spectra of $M^{n+}$-Otay + arginine + glucose systems.

Table 1. C and N added to the Na-Clay + arginine + glucose systems.

| -------- Sample---------- | | Added Arginine | | Added Glucose | | ----- Added C ----- | | ----- Added N ----- | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Weight | ----- µmol ------ | | ----- µmol ------ | | ---------- mg ---------- | | | |
| | ----- g ----- | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* |
| | 0.2475 | 125 | 505.1 | 125 | 505.1 | 18.09 | 73.08 | 7.00 | 28.29 |
| Na-SWa | 0.2471 | 125 | 505.9 | 125 | 505.9 | 18.09 | 73.20 | 7.00 | 28.34 |
| | 0.2447 | 125 | 510.8 | 125 | 510.8 | 18.09 | 73.91 | 7.00 | 28.62 |
| | 0.5008 | 250 | 499.2 | 250 | 499.2 | 36.17 | 72.23 | 14.01 | 27.97 |
| Na-Panther | 0.4918 | 250 | 508.3 | 250 | 508.3 | 36.17 | 73.55 | 14.01 | 28.48 |
| | 0.5101 | 250 | 490.1 | 250 | 490.1 | 36.17 | 70.91 | 14.01 | 27.46 |
| | 0.4978 | 250 | 502.2 | 250 | 502.2 | 36.17 | 72.67 | 14.01 | 28.14 |
| Na-Saponite | 0.5077 | 250 | 492.4 | 250 | 492.4 | 36.17 | 71.25 | 14.01 | 27.59 |
| | 0.5087 | 250 | 491.4 | 250 | 491.4 | 36.17 | 71.11 | 14.01 | 27.53 |
| | 0.5007 | 250 | 499.3 | 250 | 499.3 | 36.17 | 72.25 | 14.01 | 27.97 |
| Na-Otay | 0.4988 | 250 | 501.2 | 250 | 501.2 | 36.17 | 72.52 | 14.01 | 28.08 |
| | 0.4998 | 250 | 500.2 | 250 | 500.2 | 36.17 | 72.38 | 14.01 | 28.02 |
| Control (Arg-Glucose) | - | 250 | - | 250 | - | 36.17 | - | 14.01 | - |
| | - | 250 | - | 250 | - | 36.17 | - | 14.01 | - |

Table 2. Recovered C and N in solution from the Na-Clay + arginine + glucose systems.

| Sample | ----- Arginine-C ----- | | | ----- Glucose-C ----- | | | ----- Arginine-N ----- | | | ----- $NH_4^+$-N ----- | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *mg $g^{-1}$ clay* | ------- % ------- | | *mg $g^{-1}$ clay* | ------- % ------- | | *mg $g^{-1}$ clay* | ------- % ------- | | *mg $g^{-1}$ clay* | ------- % ------- | |
| | 11.80 | 16.15 | 14.4‡ | 32.69 | 44.73 | 41.9 | 9.09 | 32.12 | 28.6 | 4.01 | 14.2 | 16.0 |
| Na-SWa | 8.64 | 11.81 | (2.3)§ | 27.65 | 37.77 | (3.7) | 6.66 | 23.50 | (4.5) | 4.89 | 17.2 | (1.6) |
| | 11.24 | 15.21 | | 31.98 | 43.27 | | 8.66 | 30.26 | | 4.75 | 16.6 | |
| | 9.90 | 13.71 | 13.2 | 8.09 | 11.21 | 17.8 | 7.63 | 27.28 | 26.3 | 2.00 | 7.2 | 9.2 |
| Na-Panther | nd† | nd | (0.7) | nd | nd | (9.3) | nd | nd | (1.4) | nd | nd | (2.9) |
| | 9.00 | 12.68 | | 17.28 | 24.37 | | 6.93 | 25.24 | | 3.11 | 11.3 | |
| | 11.89 | 16.37 | 13.2 | 19.78 | 27.22 | 29.0 | 9.16 | 32.57 | 26.3 | 2.29 | 8.1 | 8.2 |
| Na-Saponite | 5.73 | 8.05 | (4.5) | 23.43 | 32.88 | (3.4) | 4.42 | 16.01 | (9.0) | 2.43 | 8.8 | (0.6) |
| | 10.80 | 15.19 | | 19.13 | 26.90 | | 8.32 | 30.22 | | 2.09 | 7.6 | |
| | 7.22 | 10.00 | 9.9 | 18.03 | 24.96 | 18.9 | 5.56 | 19.89 | 19.7 | 1.58 | 5.6 | 6.2 |
| Na-Otay | 7.69 | 10.60 | (0.8) | 10.65 | 14.69 | (5.4) | 5.92 | 21.09 | (1.6) | 1.58 | 5.6 | (0.9) |
| | 6.55 | 9.04 | | 12.33 | 17.04 | | 5.04 | 18.00 | | 2.04 | 7.3 | |
| Control (Arg-Glucose) | 18.20 | 50.31 | 44.7 | 15.51 | 42.89 | 38.9 | 14.02 | 100.1 | 88.9 | 1.12 | 8.0 | 7.2 |
| | 14.11 | 39.02 | (8.9) | 12.59 | 34.81 | (5.7) | 10.87 | 77.63 | (15.9) | 0.90 | 6.4 | (1.1) |

† not determined
‡ average
§ standard deviation in parenthesis

Table 3. C and N added to the Ca-Clay + arginine + glucose systems.

| -------- Sample---------- | | Added Arginine | | Added Glucose | | ----- Added C ----- | | ----- Added N ----- | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Weight | ----- µmol ------ | | ----- µmol ------ | | ---------- mg ---------- | | | |
| | ----- g ----- | *Total* | *g$^{-1}$-Clay* | *Total* | *g$^{-1}$-Clay* | *Total* | *g$^{-1}$-Clay* | *Total* | *g$^{-1}$-Clay* |
| | 0.5027 | 250 | 497.3 | 250 | 497.3 | 36.17 | 71.96 | 14.01 | 27.86 |
| Ca-SWa | 0.4810 | 250 | 519.8 | 250 | 519.8 | 36.17 | 75.20 | 14.01 | 29.12 |
| | 0.4930 | 250 | 507.1 | 250 | 507.1 | 36.17 | 73.37 | 14.01 | 28.41 |
| | 0.4914 | 250 | 508.8 | 250 | 508.8 | 36.17 | 73.61 | 14.01 | 28.50 |
| Ca-Panther | 0.5089 | 250 | 491.3 | 250 | 491.3 | 36.17 | 71.08 | 14.01 | 27.52 |
| | 0.4944 | 250 | 505.7 | 250 | 505.7 | 36.17 | 73.17 | 14.01 | 28.33 |
| | 0.5001 | 250 | 499.9 | 250 | 499.9 | 36.17 | 72.33 | 14.01 | 28.01 |
| Ca-Saponite | 0.4984 | 250 | 501.6 | 250 | 501.6 | 36.17 | 72.58 | 14.01 | 28.10 |
| | 0.4888 | 250 | 511.5 | 250 | 511.5 | 36.17 | 74.00 | 14.01 | 28.65 |
| | 0.5171 | 250 | 483.5 | 250 | 483.5 | 36.17 | 69.95 | 14.01 | 27.09 |
| Ca-Otay | 0.4886 | 250 | 511.7 | 250 | 511.7 | 36.17 | 74.03 | 14.01 | 28.66 |
| | 0.5015 | 250 | 498.5 | 250 | 498.5 | 36.17 | 72.13 | 14.01 | 27.93 |

Table 4. Recovered C and N in solution from the Ca-Clay + arginine + glucose systems.

| Sample | ----- Arginine-C ----- | | | ----- Glucose-C ----- | | | ----- Arginine-N ----- | | | ----- $NH_4^+$-N ----- | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *mg $g^{-1}$ clay* | ------- % ------- | | *mg $g^{-1}$ clay* | ------- % ------- | | *mg $g^{-1}$ clay* | ------- % ------- | | *mg $g^{-1}$ clay* | ------- % ------- | |
| | 8.71 | 12.11 | 12.5‡ | 19.42 | 26.99 | 27.1 | 6.71 | 24.1 | 24.8 | 1.51 | 5.4 | 5.4 |
| Ca-SWa | 10.2 | 13.57 | (1.0)§ | 18.09 | 24.06 | (3.1) | 7.86 | 27.0 | (1.9) | 1.55 | 5.3 | (0.1) |
| | 8.59 | 11.71 | | 22.18 | 30.23 | | 6.62 | 23.3 | | 1.58 | 5.5 | |
| | 12.19 | 16.56 | 15.5 | 33.72 | 45.80 | 48.2 | 9.39 | 33.0 | 30.9 | 1.45 | 5.1 | 5.1 |
| Ca-Panther | nd† | nd | (1.4) | nd | nd | (3.5) | nd | nd | (2.9) | nd | nd | (0.0) |
| | 10.63 | 14.52 | | 37.08 | 50.69 | | 8.19 | 28.9 | | 1.46 | 5.2 | |
| | 11.47 | 15.85 | 15.7 | 27.12 | 37.50 | 39.3 | 8.83 | 31.5 | 31.2 | 1.45 | 5.2 | 5.2 |
| Ca-Saponite | 11.09 | 15.28 | (0.3) | 29.62 | 40.81 | (1.7) | 8.54 | 30.4 | (0.7) | 1.48 | 5.3 | (0.0) |
| | 11.73 | 15.85 | | 29.35 | 39.66 | | 9.03 | 31.5 | | 1.49 | 5.2 | |
| | 10.78 | 15.41 | 12.7 | 26.06 | 37.26 | 38.2 | 8.30 | 30.7 | 25.4 | 1.50 | 5.5 | 5.4 |
| Ca-Otay | 8.56 | 11.57 | (2.3) | 26.79 | 36.19 | (2.6) | 6.60 | 23.0 | (4.6) | 1.53 | 5.3 | (0.1) |
| | 8.13 | 11.27 | | 29.62 | 41.06 | | 6.26 | 22.4 | | 1.51 | 5.4 | |

† not determined
‡ average
§ standard deviation in parenthesis

Table 5. C and N added to the Cu-Clay + arginine + glucose systems.

| -------- Sample---------- | | Added Arginine | | Added Glucose | | ----- Added C ----- | | ----- Added N ----- | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Weight | ----- µmol ------ | | ----- µmol ------ | | ---------- mg ---------- | | | |
| | ----- g ----- | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* |
| | 0.5069 | 250 | 493.2 | 250 | 493.2 | 36.17 | 71.36 | 14.01 | 27.63 |
| Cu-SWa | 0.4907 | 250 | 509.5 | 250 | 509.5 | 36.17 | 73.72 | 14.01 | 28.54 |
| | 0.5035 | 250 | 496.5 | 250 | 496.5 | 36.17 | 71.84 | 14.01 | 27.82 |
| | 0.5047 | 250 | 495.3 | 250 | 495.3 | 36.17 | 71.67 | 14.01 | 27.75 |
| Cu-Panther | 0.5121 | 250 | 488.2 | 250 | 488.2 | 36.17 | 70.64 | 14.01 | 27.35 |
| | 0.4957 | 250 | 504.3 | 250 | 504.3 | 36.17 | 72.97 | 14.01 | 28.25 |
| | 0.519 | 250 | 481.7 | 250 | 481.7 | 36.17 | 69.70 | 14.01 | 26.99 |
| Cu-Saponite | 0.5147 | 250 | 485.7 | 250 | 485.7 | 36.17 | 70.28 | 14.01 | 27.21 |
| | 0.4907 | 250 | 509.5 | 250 | 509.5 | 36.17 | 73.72 | 14.01 | 28.54 |
| | 0.4885 | 250 | 511.8 | 250 | 511.8 | 36.17 | 74.05 | 14.01 | 28.67 |
| Cu-Otay | 0.4905 | 250 | 509.7 | 250 | 509.7 | 36.17 | 73.75 | 14.01 | 28.55 |
| | 0.4877 | 250 | 512.6 | 250 | 512.6 | 36.17 | 74.17 | 14.01 | 28.72 |

Table 6. Recovered C and N in solution from the Cu-Clay + arginine + glucose systems.

| Sample | ----- Arginine-C ----- | | | ----- Glucose-C ----- | | | ----- Arginine-N ----- | | | ----- $NH_4^+$-N ----- | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *mg $g^{-1}$ clay* | ------- % ------- | | *mg $g^{-1}$ clay* | ------- % ------- | | *mg $g^{-1}$ clay* | ------- % ------- | | *mg $g^{-1}$ clay* | ------- % ------- | |
| | 1.08 | 1.51 | 1.6† | 7.18 | 10.06 | 9.4 | 0.83 | 3.0 | 3.1 | 0.12 | 0.4 | 0.4 |
| Cu-SWa | 1.15 | 1.56 | (0.1)‡ | 7.96 | 10.79 | (1.8) | 0.88 | 3.1 | (0.1) | 0.12 | 0.4 | (0.0) |
| | 1.17 | 1.63 | | 5.31 | 7.40 | | 0.90 | 3.2 | | 0.12 | 0.4 | |
| | 1.45 | 2.02 | 2.0 | 6.25 | 8.72 | 23.1 | 1.11 | 4.0 | 3.9 | 0.10 | 0.4 | 0.4 |
| Cu-Panther | 1.44 | 2.03 | (0.1) | 17.01 | 24.09 | (13.9) | 1.11 | 4.0 | (0.2) | 0.10 | 0.4 | (0.0) |
| | 1.36 | 1.87 | | 26.55 | 36.38 | | 1.05 | 3.7 | | 0.09 | 0.3 | |
| | 1.04 | 1.49 | 1.4 | 30.30 | 43.47 | 39.2 | 0.80 | 3.0 | 2.9 | 0.14 | 0.5 | 0.5 |
| Cu-Saponite | 0.95 | 1.35 | (0.1) | 29.34 | 41.74 | (5.9) | 0.73 | 2.7 | (0.1) | 0.13 | 0.5 | (0.0) |
| | 1.08 | 1.46 | | 23.92 | 32.45 | | 0.83 | 2.9 | | 0.15 | 0.5 | |
| | 0.71 | 0.96 | 0.9 | 5.73 | 7.73 | 6.0 | 0.55 | 1.9 | 1.9 | 0.08 | 0.3 | 0.3 |
| Cu-Otay | 0.63 | 0.85 | (0.1) | 2.59 | 3.51 | (2.2) | 0.48 | 1.7 | (0.1) | 0.07 | 0.3 | (0.0) |
| | 0.73 | 0.99 | | 1.93 | 6.73 | | 0.56 | 2.0 | | 0.08 | 0.3 | |

† average
‡ standard deviation in parenthesis

Table 7. C and N added to the Al-Clay + arginine + glucose systems.

| -------- Sample---------- | | Added Arginine | | Added Glucose | | ----- Added C ----- | | ----- Added N ----- | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Weight | ----- µmol ------ | | ----- µmol ------ | | ---------- mg ---------- | | | |
| | ----- g ----- | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* |
| Al-SWa | 0.2465 | 125 | 507.1 | 125 | 507.1 | 18.09 | 73.37 | 7.00 | 28.41 |
| | 0.2461 | 125 | 507.9 | 125 | 507.9 | 18.09 | 73.49 | 7.00 | 28.46 |
| | 0.4956 | 250 | 504.4 | 250 | 504.4 | 36.17 | 72.99 | 14.01 | 28.26 |
| Al-Panther | 0.5042 | 250 | 495.8 | 250 | 495.8 | 36.17 | 71.74 | 14.01 | 27.78 |
| | 0.4967 | 250 | 503.3 | 250 | 503.3 | 36.17 | 72.83 | 14.01 | 28.20 |
| | 0.4836 | 250 | 517.0 | 250 | 517.0 | 36.17 | 74.80 | 14.01 | 28.96 |
| Al-Saponite | 0.4834 | 250 | 517.2 | 250 | 517.2 | 36.17 | 74.83 | 14.01 | 28.97 |
| | 0.5173 | 250 | 483.3 | 250 | 483.3 | 36.17 | 69.93 | 14.01 | 27.07 |
| | 0.5006 | 250 | 499.4 | 250 | 499.4 | 36.17 | 72.26 | 14.01 | 27.98 |
| Al-Otay | 0.5089 | 250 | 491.3 | 250 | 491.3 | 36.17 | 71.08 | 14.01 | 27.52 |
| | 0.4984 | 250 | 501.6 | 250 | 501.6 | 36.17 | 72.58 | 14.01 | 28.10 |

Table 8. Recovered C and N in solution from the Al-Clay + arginine + glucose systems.

| Sample | ----- Arginine-C ----- | | | ----- Glucose-C ----- | | | ----- Arginine-N ----- | | | ----- $NH_4^+$-N ----- | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *mg g$^{-1}$ clay* | ------- % ------- | | *mg g$^{-1}$ clay* | ------- % ------- | | *mg g$^{-1}$ clay* | ------- % ------- | | *mg g$^{-1}$ clay* | ------- % ------- | |
| Al-SWa | 11.60 | 15.8 | 16.0† | 24.64 | 33.6 | 35.2 | 8.93 | 31.4 | 31.8 | 0.58 | 2.0 | 2.0 |
| | 11.91 | 16.2 | (0.3)‡ | 27.01 | 36.8 | (2.2) | 9.18 | 32.2 | (0.6) | 0.55 | 1.9 | (0.1) |
| | 8.03 | 11.0 | 10.8 | 27.08 | 37.1 | 35.5 | 6.19 | 21.9 | 21.6 | 0.33 | 1.2 | 1.2 |
| Al-Panther | 7.98 | 11.1 | (0.4) | 25.55 | 35.6 | (1.7) | 6.15 | 22.1 | (0.8) | 0.32 | 1.2 | (0.0) |
| | 7.58 | 10.4 | | 24.57 | 33.7 | | 5.84 | 20.7 | | 0.35 | 1.2 | |
| | 13.36 | 17.9 | 16.2 | 29.34 | 39.2 | 38.5 | 10.29 | 35.5 | 32.3 | 0.52 | 1.8 | 3.3 |
| Al-Saponite | 11.79 | 15.8 | (1.4) | 26.99 | 36.1 | (2.2) | 9.08 | 31.4 | (2.9) | 1.32 | 4.5 | (1.4) |
| | 10.55 | 15.1 | | 28.18 | 40.3 | | 8.12 | 30.0 | | 0.96 | 3.6 | |
| | 9.03 | 12.5 | 12.5 | 34.10 | 47.2 | 41.9 | 6.96 | 24.9 | 24.8 | 0.34 | 1.2 | 1.2 |
| Al-Otay | 8.85 | 12.5 | (0.0) | 29.33 | 41.3 | (4.9) | 6.82 | 24.8 | (0.1) | 0.34 | 1.2 | (0.0) |
| | 9.02 | 12.4 | | 27.13 | 37.4 | | 6.95 | 24.7 | | 0.32 | 1.1 | |

† average
‡ standard deviation in parenthesis

Table 9. C and N added to the Fe-Clay + arginine + glucose systems.

| Sample | | Added Arginine | | Added Glucose | | Added C | | Added N | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Weight | µmol | | µmol | | mg | | | |
| | g | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* | *Total* | *$g^{-1}$-Clay* |
| Fe-SWa | 0.2415 | 125 | 517.6 | 125 | 517.6 | 18.09 | 74.89 | 7.00 | 29.00 |
| | 0.2435 | 125 | 513.3 | 125 | 513.3 | 18.09 | 74.28 | 7.00 | 28.76 |
| | 0.2541 | 125 | 491.9 | 125 | 491.9 | 18.09 | 71.18 | 7.00 | 27.56 |
| Fe-Panther | 0.2400 | 125 | 520.8 | 125 | 520.8 | 18.09 | 75.36 | 7.00 | 29.18 |
| | 0.2425 | 125 | 515.5 | 125 | 515.5 | 18.09 | 74.58 | 7.00 | 28.88 |
| | 0.2400 | 125 | 520.8 | 125 | 520.8 | 18.09 | 75.36 | 7.00 | 29.18 |
| Fe-Saponite | 0.2519 | 125 | 496.2 | 125 | 496.2 | 18.09 | 71.80 | 7.00 | 27.80 |
| | 0.2461 | 125 | 507.9 | 125 | 507.9 | 18.09 | 73.49 | 7.00 | 28.46 |
| | 0.2474 | 125 | 505.3 | 125 | 505.3 | 18.09 | 73.11 | 7.00 | 28.31 |
| Fe-Otay | 0.2330 | 125 | 536.5 | 125 | 536.5 | 18.09 | 77.63 | 7.00 | 30.06 |
| | 0.2261 | 125 | 552.9 | 125 | 552.9 | 18.09 | 79.99 | 7.00 | 30.97 |

Table 10. Recovered C and N in solution from the Fe-Clay + arginine + glucose systems.

| Sample | ----- Arginine-C ----- | | | ----- Glucose-C ----- | | | ----- Arginine-N ----- | | | ----- $NH_4^+$-N ----- | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *mg g$^{-1}$ clay* | ------- % ------- | | *mg g$^{-1}$ clay* | ------- % ------- | | *mg g$^{-1}$ clay* | ------- % ------- | | *mg g$^{-1}$ clay* | ------- % ------- | |
| | 16.39 | 21.9 | 13.0† | 8.01 | 10.7 | 21.9 | 12.62 | 43.5 | 25.8 | 3.18 | 11.0 | 16.5 |
| Fe-SWa | 4.23 | 5.7 | (8.2)‡ | 31.18 | 42.0 | (17.5) | 3.26 | 11.3 | (16.3) | 7.19 | 25.0 | (7.5) |
| | 8.11 | 11.4 | | 9.17 | 12.9 | | 6.24 | 22.7 | | 3.71 | 13.5 | |
| | 13.80 | 18.3 | 19.8 | 35.47 | 47.1 | 21.2 | 10.63 | 36.4 | 39.3 | 1.43 | 4.9 | 9.2 |
| Fe-Panther | 15.67 | 21.0 | (1.4) | 7.15 | 9.6 | (22.4) | 12.07 | 41.8 | (2.7) | 3.19 | 11.0 | (3.8) |
| | 15.04 | 20.0 | | 5.25 | 7.0 | | 11.58 | 39.7 | | 3.42 | 11.7 | |
| | 16.93 | 23.6 | 22.5 | 28.26 | 39.4 | 40.5 | 13.05 | 46.9 | 44.7 | 1.33 | 4.8 | 4.6 |
| Fe-Saponite | 15.67 | 21.3 | (1.1) | 30.48 | 41.5 | (1.1) | 12.07 | 42.4 | (2.2) | 1.40 | 4.9 | (0.5) |
| | 16.41 | 22.4 | | 29.83 | 40.8 | | 12.64 | 44.7 | | 1.13 | 4.0 | |
| | 17.20 | 22.2 | 22.2 | 32.88 | 42.4 | 42.2 | 13.25 | 44.1 | 44.2 | 0.99 | 3.3 | 3.4 |
| | 17.84 | 22.3 | (0.1) | 33.58 | 42.0 | (0.3) | 13.74 | 44.4 | (0.2) | 1.06 | 3.4 | (0.1) |

† average
‡ standard deviation in parenthesis

Table 11. C and N added to the goethite + arginine + glucose systems.

| -------- Sample---------- | | Added Arginine | | Added Glucose | | ----- Added C ----- | | ----- Added N ----- | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Weight | ----- µmol ------ | | ----- µmol ------ | | ---------- mg ---------- | | | |
| | ----- g ----- | *Total* | *$g^{-1}$-goethite* | *Total* | *$g^{-1}$-goethite* | *Total* | *$g^{-1}$-goethite* | *Total* | *$g^{-1}$-goethite* |
| | 0.4973 | 250 | 502.7 | 250 | 502.7 | 36.17 | 72.74 | 14.01 | 28.16 |
| Goe-00 | 0.5073 | 250 | 492.8 | 250 | 492.8 | 36.17 | 71.31 | 14.01 | 27.61 |
| | 0.5140 | 250 | 486.4 | 250 | 486.4 | 36.17 | 70.38 | 14.01 | 27.25 |
| | 0.4924 | 250 | 507.7 | 250 | 507.7 | 36.17 | 73.46 | 14.01 | 28.44 |
| Goe-20 | 0.4957 | 250 | 504.3 | 250 | 504.3 | 36.17 | 72.97 | 14.01 | 28.25 |
| | 0.4948 | 250 | 505.3 | 250 | 505.3 | 36.17 | 73.11 | 14.01 | 28.31 |
| | 0.4775 | 250 | 523.6 | 250 | 523.6 | 36.17 | 75.76 | 14.01 | 29.33 |
| Goe-52 | 0.4760 | 250 | 525.2 | 250 | 525.2 | 36.17 | 75.99 | 14.01 | 29.42 |
| | 0.5001 | 250 | 499.9 | 250 | 499.9 | 36.17 | 72.33 | 14.01 | 28.01 |
| | 0.4844 | 250 | 516.1 | 250 | 516.1 | 36.17 | 74.68 | 14.01 | 28.91 |
| Goe-94 | 0.4732 | 250 | 528.3 | 250 | 528.3 | 36.17 | 76.44 | 14.01 | 29.60 |
| | 0.4895 | 250 | 510.7 | 250 | 510.7 | 36.17 | 73.90 | 14.01 | 28.61 |

Table 12. Recovered C and N in solution from the goethite + arginine + glucose systems.

| Sample | ----- Arginine-C ----- | | | ----- Glucose-C ----- | | | ----- Arginine-N ----- | | | ----- $NH_4^+$-N ----- | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *mg g$^{-1}$ goethite* | ------- % ------- | | *mg g$^{-1}$ goethite* | ------- % ------- | | *mg g$^{-1}$ goethite* | ------- % ------- | | *mg g$^{-1}$ goethite* | ------- % ------- | |
| | 19.86 | 27.3 | 29.3‡ | 31.14 | 42.8 | 40.4 | 15.30 | 54.3 | 58.2 | 2.21 | 7.8 | 7.7 |
| Goe-00 | 21.56 | 30.2 | (1.7)§ | 32.64 | 45.8 | (7.0) | 16.61 | 60.1 | (3.4) | 2.02 | 7.3 | (0.4) |
| | 21.30 | 30.3 | | 22.88 | 32.5 | | 16.41 | 60.2 | | 2.18 | 8.0 | |
| | 22.76 | 31.0 | 30.1 | 30.77 | 41.9 | 41.6 | 17.53 | 61.6 | 59.9 | 2.11 | 7.4 | 7.2 |
| Goe-20 | nd† | nd | (1.2) | nd | nd | (0.4) | nd | nd | (2.5) | nd | nd | (0.3) |
| | 21.36 | 29.2 | | 30.20 | 41.3 | | 16.45 | 58.1 | | 1.97 | 7.0 | |
| | 21.47 | 28.3 | 29.8 | 28.07 | 37.1 | 37.5 | 16.54 | 56.4 | 59.4 | 2.07 | 7.1 | 7.4 |
| Goe-52 | 22.69 | 29.9 | (1.5) | 28.07 | 36.9 | (0.8) | 17.48 | 59.4 | (3.0) | 2.18 | 7.4 | (0.4) |
| | 22.66 | 31.3 | | 27.80 | 38.4 | | 17.46 | 62.3 | | 2.19 | 7.8 | |
| | 21.72 | 29.1 | 29.5 | 29.32 | 39.3 | 38.6 | 16.73 | 57.9 | 58.8 | 2.26 | 7.8 | 7.5 |
| Goe-94 | 22.86 | 29.9 | (0.4) | 29.63 | 38.8 | (0.8) | 17.61 | 59.5 | (0.8) | 2.12 | 7.2 | (0.3) |
| | 21.86 | 29.6 | | 27.91 | 37.8 | | 16.84 | 58.9 | | 2.11 | 7.4 | |

† not determined
‡ average
§ standard deviation in parenthesis

Table 13. Soluble organic C in clay + arginine + glucose systems.[†]

| Clay System | weight | C found Solution | | | |
|---|---|---|---|---|---|
| | | Total | $g^{-1}$ clay | | |
| | — g — | mg | | % | |
| Na-SWa | 0.2475 | 16.89 | 68.2 | 93.4 | 90.0 |
| | 0.2471 | 16.12 | 65.2 | 89.1 | (3.0)[‡] |
| | 0.2447 | 15.85 | 64.8 | 87.6 | |
| Na-Panther | 0.5008 | 14.55 | 29.0 | 40.2 | 42.1 |
| | 0.4918 | 14.57 | 29.6 | 40.3 | (3.2) |
| | 0.5101 | 16.54 | 32.4 | 45.7 | |
| Na-Saponite | 0.4978 | 24.70 | 49.6 | 68.3 | 66.8 |
| | 0.5077 | 23.48 | 46.3 | 64.9 | (1.7) |
| | 0.5087 | 24.25 | 47.7 | 67.0 | |
| Na-Otay | 0.5007 | 25.82 | 51.6 | 71.4 | 66.2 |
| | 0.4988 | 23.37 | 46.9 | 64.6 | (4.6) |
| | 0.4998 | 22.68 | 45.4 | 62.7 | |
| Ca-SWa | 0.5027 | 26.65 | 53.0 | 73.7 | 69.4 |
| | 0.4810 | 21.91 | 45.6 | 60.6 | (7.7) |
| | 0.4930 | 26.81 | 54.4 | 74.1 | |
| Ca-Panther | 0.4914 | 27.39 | 55.7 | 75.7 | 70.8 |
| | 0.5089 | 22.47 | 44.2 | 62.1 | (7.5) |
| | 0.4944 | 26.94 | 54.5 | 74.5 | |
| Ca-Saponite | 0.5001 | 27.82 | 55.6 | 76.9 | 77.5 |
| | 0.4984 | 28.11 | 56.4 | 77.7 | (0.5) |
| | 0.4888 | 28.16 | 57.6 | 77.9 | |
| Ca-Otay | 0.5171 | 27.36 | 52.9 | 75.6 | 75.6 |
| | 0.4886 | 27.34 | 55.9 | 75.6 | (0.1) |
| | 0.5015 | 27.39 | 54.6 | 75.7 | |
| Cu-SWa | 0.5069 | 22.10 | 43.6 | 61.1 | 61.3 |
| | 0.4907 | 22.47 | 45.8 | 62.1 | (0.8) |
| | 0.5035 | 21.94 | 43.6 | 60.6 | |
| Cu-Panther | 0.5047 | 22.23 | 44.0 | 61.5 | 61.5 |
| | 0.5121 | 22.36 | 43.7 | 61.8 | (0.3) |
| | 0.4957 | 22.18 | 44.7 | 61.3 | |

Table 13. Soluble organic C in clay + arginine + glucose systems. *(continuation)*

| Clay System | weight | C found Solution | | | |
|---|---|---|---|---|---|
| | | Total | $g^{-1}$ clay | | |
| | *g* | *mg* | | % | |
| Cu-Saponite | 0.5190 | 21.51 | 41.5 | 59.5 | 59.5 |
| | 0.5147 | 21.97 | 42.7 | 60.7 | (1.1) |
| | 0.4907 | 21.14 | 43.1 | 58.4 | |
| Cu-Otay | 0.4885 | 22.68 | 46.4 | 62.7 | 61.3 |
| | 0.4905 | 21.89 | 44.6 | 60.5 | (1.2) |
| | 0.4877 | 21.94 | 45.0 | 60.6 | |
| Al-SWa | 0.2465 | 15.10 | 61.3 | 83.5 | 84.0 |
| | 0.2461 | 15.26 | 62.0 | 84.4 | (0.6) |
| Al-Panther | 0.4956 | 19.47 | 39.3 | 53.8 | 54.5 |
| | 0.5042 | 19.71 | 39.1 | 54.5 | (0.7) |
| | 0.4967 | 19.94 | 40.2 | 55.1 | |
| Al-Saponite | 0.4836 | 26.67 | 55.2 | 73.7 | 74.6 |
| | 0.4834 | 26.81 | 55.5 | 74.1 | (1.3) |
| | 0.5173 | 27.52 | 53.2 | 76.1 | |
| Al-Otay | 0.5006 | 27.47 | 54.9 | 75.9 | 73.4 |
| | 0.5089 | 26.01 | 51.1 | 71.9 | (2.2) |
| | 0.4984 | 26.19 | 52.6 | 72.4 | |
| Fe-SWa | 0.2415 | 8.90 | 36.9 | 49.2 | 54.0 |
| | 0.2435 | 10.44 | 42.9 | 57.7 | (4.4) |
| | 0.2541 | 9.98 | 39.3 | 55.2 | |
| Fe-Panther | 0.2400 | 14.60 | 60.8 | 80.7 | 61.5 |
| | 0.2425 | 9.46 | 39.0 | 52.3 | (16.6) |
| | 0.2400 | 9.34 | 38.9 | 51.6 | |
| Fe-Saponite | 0.2519 | 14.99 | 59.5 | 82.9 | 80.6 |
| | 0.2461 | 13.58 | 55.2 | 75.1 | (4.8) |
| | 0.2474 | 15.14 | 61.2 | 83.7 | |
| Fe-Otay | 0.2330 | 14.20 | 61.0 | 78.5 | 80.9 |
| | 0.2260 | 15.05 | 66.6 | 83.2 | (3.3) |

† soluble organic C determined by the $K_2Cr_2O_7$ method.
‡ Standard deviation in parenthesis.

Table 14. Soluble organic C in goethite + arginine + glucose systems.†

| Goethite | weight | C found Solution | | | |
|---|---|---|---|---|---|
| | | Total | $g^{-1}$ goethite | | |
| | *g* | *mg* | | % | |
| Goe-00 | 0.4973 | 35.1 | 70.6 | 97.1 | 98.3 |
| | 0.5073 | 36.2 | 71.4 | 100.1 | (1.6)‡ |
| | 0.5140 | 35.4 | 68.8 | 97.8 | |
| Goe-20 | 0.4924 | 33.4 | 67.9 | 92.5 | 91.4 |
| | 0.4957 | 32.5 | 65.6 | 89.9 | (1.4) |
| | 0.4948 | 33.2 | 67.2 | 91.9 | |
| Goe-52 | 0.4775 | 34.7 | 72.6 | 95.9 | 95.5 |
| | 0.4760 | 34.5 | 72.5 | 95.4 | (0.3) |
| | 0.5001 | 34.5 | 68.9 | 95.3 | |
| Goe-94 | 0.4844 | 33.7 | 69.5 | 93.0 | 94.2 |
| | 0.4732 | 34.3 | 72.5 | 94.8 | (1.0) |
| | 0.4895 | 34.2 | 70.0 | 94.7 | |

† soluble organic C determined by the $K_2Cr_2O_7$ method.
‡ Standard deviation in parenthesis.

Table 15. Evolved $CO_2$-C in clay + arginine + glucose systems.

| Clay System | weight | Added C | Evolved $CO_2$-C | | |
|---|---|---|---|---|---|
| | | | $g^{-1}$ clay | | |
| | *g* | *mg* | | % | |
| | 0.2475 | 73.08 | 0.25 | 0.34 | 0.5 |
| Na-SWa | 0.2471 | 73.20 | 0.63 | 0.87 | (0.4)† |
| | 0.2447 | 73.91 | 0.15 | 0.20 | |
| | 0.5008 | 72.23 | 1.10 | 1.52 | 1.5 |
| Na-Panther | 0.4918 | 73.55 | 0.95 | 1.29 | (0.2) |
| | 0.5101 | 70.91 | 1.21 | 1.70 | |
| | 0.4978 | 72.67 | 0.01 | 0.02 | 0.1 |
| Na-Saponite | 0.5077 | 71.25 | 0.11 | 0.15 | (0.1) |
| | 0.5087 | 71.11 | 0.00 | 0.00 | |
| | 0.5007 | 72.25 | 0.00 | 0.00 | 0.0 |
| Na-Otay | 0.4988 | 72.52 | 0.00 | 0.00 | (0.0) |
| | 0.4998 | 72.38 | 0.00 | 0.00 | |
| | 0.5027 | 71.96 | 0.04 | 0.05 | 0.1 |
| Ca-SWa | 0.4810 | 75.20 | 0.12 | 0.17 | (0.1) |
| | 0.4930 | 73.37 | 0.04 | 0.05 | |
| | 0.4914 | 73.61 | 0.00 | 0.00 | 1.0 |
| Ca-Panther | 0.5089 | 71.08 | 2.09 | 2.95 | (1.7) |
| | 0.4944 | 73.17 | 0.01 | 0.02 | |
| | 0.5001 | 72.33 | 0.00 | 0.00 | 0.0 |
| Ca-Saponite | 0.4984 | 72.58 | 0.00 | 0.00 | (0.0) |
| | 0.4888 | 74.00 | 0.00 | 0.00 | |
| | 0.5171 | 69.95 | 0.00 | 0.00 | 0.0 |
| Ca-Otay | 0.4886 | 74.03 | 0.00 | 0.00 | (0.0) |
| | 0.5015 | 72.13 | 0.00 | 0.00 | |
| | 0.5069 | 71.36 | 0.00 | 0.00 | 0.0 |
| Cu-SWa | 0.4907 | 73.72 | 0.00 | 0.00 | (0.0) |
| | 0.5035 | 71.84 | 0.00 | 0.00 | |
| | 0.5047 | 71.67 | 0.00 | 0.00 | 0.0 |
| Cu-Panther | 0.5121 | 70.64 | 0.00 | 0.00 | (0.0) |
| | 0.4957 | 72.97 | 0.00 | 0.00 | |

Table 15. Evolved $CO_2$-C in clay + arginine + glucose systems. *(continuation)*

| Clay System | weight | Added C | Evolved $CO_2$-C | | |
|---|---|---|---|---|---|
| | | | $g^{-1}$ clay | | |
| | *g* | *mg* | | % | |
| Cu-Saponite | 0.5190 | 69.70 | 0.01 | 0.02 | 0.0 |
| | 0.5147 | 70.28 | 0.01 | 0.02 | (0.0) |
| | 0.4907 | 73.72 | 0.01 | 0.02 | |
| Cu-Otay | 0.4885 | 74.05 | 0.01 | 0.01 | 0.0 |
| | 0.4905 | 73.75 | 0.01 | 0.01 | (0.0) |
| | 0.4877 | 74.17 | 0.00 | 0.01 | |
| Al-SWa | 0.2465 | 73.37 | 0.04 | 0.05 | 0.0 |
| | 0.2461 | 73.49 | 0.02 | 0.03 | (0.0) |
| Al-Panther | 0.4956 | 72.99 | 0.12 | 0.17 | 0.1 |
| | 0.5042 | 71.74 | 0.00 | 0.00 | (0.1) |
| | 0.4967 | 72.83 | 0.00 | 0.00 | |
| Al-Saponite | 0.4836 | 74.80 | 0.16 | 0.22 | 0.3 |
| | 0.4834 | 74.83 | 0.37 | 0.50 | (0.2) |
| | 0.5173 | 69.93 | 0.13 | 0.19 | |
| Al-Otay | 0.5006 | 72.26 | 0.00 | 0.00 | 0.0 |
| | 0.5089 | 71.08 | 0.00 | 0.00 | (0.0) |
| | 0.4984 | 72.58 | 0.00 | 0.00 | |
| Fe-SWa | 0.2415 | 74.89 | 2.18 | 2.91 | 2.5 |
| | 0.2435 | 74.28 | 2.01 | 2.71 | (0.5) |
| | 0.2541 | 71.18 | 1.40 | 1.96 | |
| Fe-Panther | 0.2400 | 75.36 | 0.28 | 0.38 | 1.9 |
| | 0.2425 | 74.58 | 2.10 | 2.81 | (1.3) |
| | 0.2400 | 75.36 | 1.94 | 2.58 | |
| Fe-Saponite | 0.2519 | 71.80 | 0.21 | 0.29 | 0.3 |
| | 0.2461 | 73.49 | 0.23 | 0.32 | (0.0) |
| | 0.2474 | 73.11 | 0.20 | 0.27 | |
| Fe-Otay | 0.2330 | 77.63 | 0.17 | 0.22 | 0.3 |
| | 0.2260 | 79.99 | 0.26 | 0.33 | (0.1) |

† Standard deviation in parenthesis.

Table 16. Evolved $CO_2$-C in goethite + arginine + glucose systems.

| Goethite | weight | Added C | Evolved $CO_2$-C | | |
|---|---|---|---|---|---|
| | | | $g^{-1}$ goethite | | |
| | *g* | *mg* | | % | |
| Goe-00 | 0.4973 | 72.74 | 0.01 | 0.01 | 0.0 |
| | 0.5073 | 71.31 | 0.01 | 0.01 | (0.0)† |
| | 0.5140 | 70.38 | 0.01 | 0.01 | |
| Goe-20 | 0.4924 | 73.46 | 0.01 | 0.01 | |
| | 0.4957 | 72.97 | 0.01 | 0.01 | 0.0 |
| | 0.4948 | 73.11 | 0.01 | 0.01 | (0.0) |
| Goe-52 | 0.4775 | 75.76 | 0.01 | 0.01 | 0.0 |
| | 0.4760 | 75.99 | 0.01 | 0.01 | (0.0) |
| | 0.5001 | 72.33 | 0.01 | 0.01 | |
| Goe-94 | 0.4844 | 74.68 | 0.01 | 0.01 | 0.0 |
| | 0.4732 | 76.44 | 0.01 | 0.01 | |
| | 0.4895 | 73.90 | 0.01 | 0.01 | (0.0) |

† Standard deviation in parenthesis.

# APPENDIX D

## RAW DATA FROM CHAPTER 5

(p. 194-202)

Table 1. C and N added to the goethite + diglycine + glucose systems.

| -------- Sample---------- | | Added Diglycine | | Added Glucose | | ----- Added C ----- | | ----- Added N ----- | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Weight | ----- µmol ------ | | ----- µmol ------ | | ---------- mg ---------- | | | |
| | ----- g ----- | *Total* | *$g^{-1}$-goethite* | *Total* | *$g^{-1}$-goethite* | *Total* | *$g^{-1}$-goethite* | *Total* | *$g^{-1}$-goethite* |
| | 0.5081 | 250 | 492.0 | 250 | 492.0 | 30.00 | 59.05 | 7.00 | 13.78 |
| Goe-00 | 0.5024 | 250 | 497.6 | 250 | 497.6 | 30.00 | 59.72 | 7.00 | 13.94 |
| | 0.5115 | 250 | 488.8 | 250 | 488.8 | 30.00 | 58.65 | 7.00 | 13.69 |
| | 0.4894 | 250 | 510.8 | 250 | 510.8 | 30.00 | 61.30 | 7.00 | 14.31 |
| Goe-20 | 0.4860 | 250 | 514.4 | 250 | 514.4 | 30.00 | 61.73 | 7.00 | 14.41 |
| | 0.4983 | 250 | 501.7 | 250 | 501.7 | 30.00 | 60.21 | 7.00 | 14.05 |
| | 0.4737 | 250 | 527.8 | 250 | 527.8 | 30.00 | 63.33 | 7.00 | 14.78 |
| Goe-51 | 0.4747 | 250 | 526.6 | 250 | 526.6 | 30.00 | 63.20 | 7.00 | 14.75 |
| | 0.4980 | 250 | 502.0 | 250 | 502.0 | 30.00 | 60.24 | 7.00 | 14.06 |
| | 0.4912 | 250 | 509.0 | 250 | 509.0 | 30.00 | 61.08 | 7.00 | 14.25 |
| Goe-94 | 0.4960 | 250 | 504.0 | 250 | 504.0 | 30.00 | 60.49 | 7.00 | 14.12 |
| | 0.4812 | 250 | 519.5 | 250 | 519.5 | 30.00 | 62.35 | 7.00 | 14.55 |
| Control (Diglycine-Glucose) | - | 250 | - | 250 | - | 30.00 | | 7.00 | - |
| | - | 250 | - | 250 | - | 30.00 | | 7.00 | - |

Table 2. Recovered C in solution from the goethite + diglycine + glucose systems.

| Sample | ----- Diglycine-C ----- | | | ----- Glycine-C ----- | | | ----- Glucose-C ----- | | |
|---|---|---|---|---|---|---|---|---|---|
| | *mg g$^{-1}$ goethite* | ------ % ------ | | *mg g$^{-1}$ goethite* | ------ % ------ | | *mg g$^{-1}$ goethite* | ------ % ------ | |
| | 20.0 | 33.9 | 34.1† | 0.5 | 0.8 | 0.8 | 34.8 | 58.9 | 58.3 |
| Goe-00 | 19.4 | 32.5 | (1.8)‡ | 0.4 | 0.7 | (0.0) | 34.4 | 57.6 | (0.6) |
| | 21.1 | 36.0 | | 0.5 | 0.8 | | 34.3 | 58.5 | |
| | 15.8 | 25.8 | 28.2 | 0.6 | 0.9 | 0.9 | 32.9 | 53.7 | 54.5 |
| Goe-20 | 15.1 | 24.4 | (5.3) | 0.6 | 0.9 | (0.1) | 33.2 | 53.7 | (1.3) |
| | 20.6 | 34.2 | | 0.5 | 0.8 | | 33.7 | 55.9 | |
| | 24.3 | 38.3 | 37.5 | 0.4 | 0.7 | 0.7 | 35.9 | 56.7 | 56.2 |
| Goe-51 | 21.8 | 34.5 | (2.8) | 0.4 | 0.6 | (0.2) | 37.0 | 58.5 | (2.5) |
| | 24.0 | 39.8 | | 0.6 | 0.9 | | 32.2 | 53.5 | |
| | 21.9 | 35.8 | 35.1 | 0.7 | 1.1 | 1.0 | 30.6 | 50.0 | 51.4 |
| Goe-94 | 21.8 | 36.0 | (1.4) | 0.6 | 1.0 | (0.1) | 32.0 | 52.9 | (1.4) |
| | 20.9 | 33.5 | | 0.5 | 0.8 | | 32.0 | 51.3 | |
| Control (Diglycine-Glucose) | 11.5 | 38.3 | 37.2 | 0.3 | 1.1 | 1.0 | 17.6 | 58.5 | 57.8 |
| | 10.8 | 36.1 | (1.5) | 0.3 | 0.9 | (0.1) | 17.1 | 57.1 | (1.0) |

† average
‡ standard deviation in parenthesis

Table 3. Recovered N in solution from the goethite + diglycine + glucose systems.

| Sample | ----- Diglycine-N ----- | | | ----- Glycine-N ----- | | | ----- $NH_4^+$-N ----- | | |
|---|---|---|---|---|---|---|---|---|---|
| | *mg $g^{-1}$ goethite* | ------ % ------ | | *mg $g^{-1}$ goethite* | ------ % ------ | | *mg $g^{-1}$ goethite* | ------ % ------ | |
| | 11.7 | 84.8 | 85.3† | 0.3 | 2.0 | 2.0 | 0.5 | 3.8 | 3.8 |
| Goe-00 | 11.3 | 81.1 | (4.5)‡ | 0.3 | 1.9 | (0.1) | 0.5 | 3.6 | (0.2) |
| | 12.3 | 90.0 | | 0.3 | 2.1 | | 0.6 | 4.0 | |
| | 9.2 | 64.5 | 70.4 | 0.3 | 2.3 | 2.2 | 0.4 | 2.9 | 2.8 |
| Goe-20 | 8.8 | 61.1 | (13.3) | 0.3 | 2.3 | (0.2) | 0.4 | 2.7 | (0.1) |
| | 12.0 | 85.6 | | 0.3 | 2.0 | | 0.4 | 2.7 | |
| | 14.1 | 95.7 | 93.8 | 0.3 | 1.7 | 1.9 | 0.5 | 3.1 | 3.3 |
| Goe-51 | 12.7 | 86.2 | (6.9) | 0.2 | 1.5 | (0.5) | 0.4 | 2.8 | (0.7) |
| | 14.0 | 99.6 | | 0.3 | 2.4 | | 0.6 | 4.1 | |
| | 12.7 | 89.4 | 87.7 | 0.4 | 2.7 | 2.4 | 0.7 | 4.9 | 4.4 |
| Goe-94 | 12.7 | 89.9 | (3.4) | 0.3 | 2.4 | (0.3) | 0.6 | 4.5 | (0.5) |
| | 12.2 | 83.8 | | 0.3 | 2.1 | | 0.6 | 4.0 | |
| Control (Diglycine-Glucose) | 6.7 | 95.7 | 93.1 | 0.2 | 2.3 | 2.1 | 0.2 | 3.0 | 2.8 |
| | 6.3 | 90.4 | (3.8) | 0.1 | 1.9 | (0.3) | 0.2 | 2.7 | (0.2) |

† average
‡ standard deviation in parenthesis

Table 4. C and N added to the goethite + triglycine + glucose systems.

| -------- Sample---------- | | Added Triglycine | | Added Glucose | | ----- Added C ----- | | ----- Added N ----- | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Weight | ----- µmol ------ | | ----- µmol ------ | | ---------- mg ---------- | | | |
| | ----- g ----- | *Total* | *$g^{-1}$-goethite* | *Total* | *$g^{-1}$-goethite* | *Total* | *$g^{-1}$-goethite* | *Total* | *$g^{-1}$-goethite* |
| | 0.4887 | 250 | 511.6 | 250 | 511.6 | 36.00 | 73.66 | 10.50 | 21.49 |
| Goe-00 | 0.5025 | 250 | 497.5 | 250 | 497.5 | 36.00 | 71.64 | 10.50 | 20.90 |
| | 0.5089 | 250 | 491.3 | 250 | 491.3 | 36.00 | 70.74 | 10.50 | 20.63 |
| | 0.4950 | 250 | 505.1 | 250 | 505.1 | 36.00 | 72.72 | 10.50 | 21.21 |
| Goe-20 | 0.4842 | 250 | 516.3 | 250 | 516.3 | 36.00 | 74.34 | 10.50 | 21.69 |
| | 0.4909 | 250 | 509.3 | 250 | 509.3 | 36.00 | 73.33 | 10.50 | 21.39 |
| | 0.4887 | 250 | 511.6 | 250 | 511.6 | 36.00 | 73.66 | 10.50 | 21.49 |
| Goe-51 | 0.4740 | 250 | 527.4 | 250 | 527.4 | 36.00 | 75.94 | 10.50 | 22.15 |
| | 0.4812 | 250 | 519.5 | 250 | 519.5 | 36.00 | 74.81 | 10.50 | 21.82 |
| | 0.4962 | 250 | 503.8 | 250 | 503.8 | 36.00 | 72.55 | 10.50 | 21.16 |
| Goe-94 | 0.4836 | 250 | 517.0 | 250 | 517.0 | 36.00 | 74.44 | 10.50 | 21.71 |
| | 0.4922 | 250 | 507.9 | 250 | 507.9 | 36.00 | 73.14 | 10.50 | 21.33 |
| | - | 250 | - | 250 | - | 36.00 | - | 10.50 | - |
| Control (triglycine-glucose) | - | 250 | - | 250 | - | 36.00 | - | 10.50 | - |
| | - | 250 | - | 250 | - | 36.00 | - | 10.50 | - |

Table 5. Recovered C in solution from the goethite + triglycine + glucose systems.

| Sample | ----- Triglycine-C ----- | | | ----- Diglycine-C ----- | | | ----- Glycine-C ----- | | | ----- Glucose-C ----- | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *mg g$^{-1}$ goethite* | ------ % ------ | | *mg g$^{-1}$ goethite* | ------ % ------ | | *mg g$^{-1}$ goethite* | ------ % ------ | | *mg g$^{-1}$ goethite* | ------ % ------ | |
| | 30.7 | 44.2 | 48.2† | 2.1 | 2.8 | 2.5 | 0.5 | 0.6 | 0.6 | 32.6 | 44.2 | 48.2 |
| Goe-00 | 27.3 | 53.1 | (4.5)‡ | 1.6 | 2.2 | (0.3) | 0.4 | 0.5 | (0.1) | 38.0 | 53.1 | (4.5) |
| | 29.5 | 47.2 | | 1.6 | 2.3 | | 0.4 | 0.6 | | 33.4 | 47.2 | |
| | 25.5 | 50.0 | 46.1 | 2.3 | 3.1 | 3.8 | 0.5 | 0.7 | 0.7 | 36.3 | 50.0 | 46.1 |
| Goe-20 | 33.4 | 44.9 | (3.4) | 3.2 | 4.3 | (0.6) | 0.6 | 0.7 | (0.0) | 33.4 | 44.9 | (3.4) |
| | 33.6 | 43.5 | | 2.9 | 4.0 | | 0.6 | 0.8 | | 31.9 | 43.5 | |
| | 30.1 | 46.2 | 45.3 | 2.8 | 3.9 | 4.2 | 0.5 | 0.7 | 0.8 | 34.0 | 46.2 | 45.3 |
| Goe-51 | 34.1 | 42.8 | (2.2) | 2.9 | 3.8 | (0.6) | 0.5 | 0.7 | (0.1) | 32.5 | 42.8 | (2.2) |
| | 28.0 | 47.0 | | 3.7 | 4.9 | | 0.7 | 0.9 | | 35.2 | 47.0 | |
| | 27.8 | 53.0 | 50.8 | 3.7 | 5.1 | 5.0 | 0.8 | 1.1 | 1.0 | 38.4 | 53.0 | 50.8 |
| Goe-94 | 25.0 | 48.7 | (3.1) | 3.6 | 4.9 | (0.1) | 0.7 | 1.0 | (0.1) | 35.6 | 48.7 | (3.1) |
| | 20.2 | 59.4 | 56.1 | 0.1 | 0.1 | 0.1 | 0.6 | 1.8 | 1.7 | 17.8 | 49.3 | 46.3 |
| Control (triglycine-glucose) | 1.7 | 52.7 | (4.7) | 0.1 | 0.1 | (0.0) | 0.0 | 1.7 | (0.0) | 15.6 | 43.3 | (4.3) |

† average
‡ standard deviation in parenthesis

Table 6. Recovered N in solution from the goethite + triglycine + glucose systems.

| Sample | ----- Triglycine-N ----- | | | ----- Diglycine-N ----- | | | ----- Glycine-N ----- | | | ----- $NH_4^+$-N ----- | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *mg g$^{-1}$ goethite* | ------ % ------ | | *mg g$^{-1}$ goethite* | ------ % ------ | | *mg g$^{-1}$ goethite* | ------ % ------ | | *mg g$^{-1}$ goethite* | ------ % ------ | |
| | 17.9 | 83.3 | 81.0† | 1.2 | 5.7 | 4.9 | 0.3 | 1.3 | 1.1 | 0.7 | 3.1 | 2.8 |
| Goe-00 | 15.9 | 76.1 | (4.2)‡ | 0.9 | 4.5 | (0.6) | 0.2 | 1.0 | (0.1) | 0.5 | 2.5 | (0.3) |
| | 17.2 | 83.5 | | 1.0 | 4.7 | | 0.2 | 1.1 | | 0.6 | 2.7 | |
| | 14.9 | 70.0 | 83.8 | 1.3 | 6.3 | 7.6 | 0.3 | 1.4 | 1.5 | 0.5 | 2.5 | 2.7 |
| Goe-20 | 19.5 | 89.7 | (12.0) | 1.8 | 8.5 | (1.2) | 0.3 | 1.5 | (0.1) | 0.6 | 2.9 | (0.2) |
| | 19.6 | 91.7 | | 1.7 | 7.9 | | 0.3 | 1.5 | | 0.6 | 2.7 | |
| | 17.6 | 81.7 | 82.2 | 1.7 | 7.7 | 8.4 | 0.3 | 1.4 | 1.5 | 0.6 | 2.6 | 2.6 |
| Goe-51 | 19.9 | 89.9 | (7.5) | 1.7 | 7.7 | (1.2) | 0.3 | 1.3 | (0.3) | 0.7 | 3.0 | (0.3) |
| | 16.3 | 74.9 | | 2.1 | 9.8 | | 0.4 | 1.8 | | 0.5 | 2.3 | |
| | 16.2 | 76.7 | 72.5 | 2.2 | 10.2 | 10.0 | 0.5 | 2.2 | 2.1 | 0.8 | 4.0 | 3.4 |
| Goe-94 | 14.6 | | (6.0) | 2.1 | | (0.3) | 0.4 | | (0.1) | 0.6 | | (0.8) |
| | 12.5 | 118.8 | 112.1 | 0.1 | 0.1 | 0.1 | 0.4 | 3.5 | 3.4 | 0.7 | 6.6 | 6.1 |
| Control (triglycine-glucose) | 11.1 | 105.4 | (9.4) | 0.1 | 0.1 | (0.0) | 0.4 | 3.4 | (0.1) | 0.6 | 5.6 | (0.7) |

† average
‡ standard deviation in parenthesis

Table 7. Soluble organic C in goethite + peptide + glucose systems.†

| Goethite | weight | C found Solution | | | |
|---|---|---|---|---|---|
| | | Total | $g^{-1}$ goehtite | | |
| | — g — | — mg — | | — % — | |
| ***Di-glycine*** | | | | | |
| | 0.5081 | 31.20 | 61.40 | 104.0 | 102.9 |
| Goe-00 | 0.5024 | 30.74 | 61.20 | 102.5 | (1.0)‡ |
| | 0.5115 | 30.66 | 59.95 | 102.2 | |
| | 0.4894 | 30.45 | 62.22 | 101.5 | 101.9 |
| Goe-20 | 0.4860 | 30.82 | 63.43 | 102.7 | (0.7) |
| | 0.4983 | 30.42 | 61.05 | 101.4 | |
| | 0.4737 | 30.50 | 64.39 | 101.7 | 103.0 |
| Goe-51 | 0.4747 | 30.85 | 64.99 | 102.8 | (1.4) |
| | 0.4980 | 31.36 | 62.97 | 104.5 | |
| | 0.4912 | 29.86 | 60.79 | 99.5 | 100.6 |
| Goe-94 | 0.4960 | 30.05 | 60.58 | 100.2 | (1.4) |
| | 0.4812 | 30.66 | 63.72 | 102.2 | |
| ***Triglycine*** | | | | | |
| | 0.4887 | 34.35 | 70.30 | 95.4 | 97.9 |
| Goe-00 | 0.5025 | 35.88 | 71.40 | 99.7 | (2.2) |
| | 0.5089 | 35.50 | 69.76 | 98.6 | |
| | 0.4950 | 36.14 | 73.02 | 100.4 | 100.0 |
| Goe-20 | 0.4842 | 35.88 | 74.10 | 99.7 | (0.4) |
| | 0.4909 | 35.93 | 73.19 | 99.8 | |
| | 0.4887 | 35.80 | 73.25 | 99.4 | 100.7 |
| Goe-51 | 0.4740 | 36.68 | 77.38 | 101.9 | (1.2) |
| | 0.4812 | 36.23 | 75.28 | 100.6 | |
| | 0.4962 | 34.78 | 70.10 | 96.6 | 96.5 |
| Goe-94 | 0.4836 | 34.83 | 72.03 | 96.8 | (0.4) |
| | 0.4911 | 34.57 | 70.39 | 96.0 | |

† soluble organic C determined by the $K_2Cr_2O_7$ method.
‡ Standard deviation in parenthesis.

Table 7. Evolved $CO_2$-C in goethite + peptide + glucose systems.

| Goehtite | weight | Added C | Evolved $CO_2$-C | | |
|---|---|---|---|---|---|
| | | | $g^{-1}$ goethite | | |
| | *g* | *mg* | | % | |
| ***Di-glycine*** | | | | | |
| | 0.5081 | 59.05 | 0.57 | 1.0 | 0.9 |
| Goe-00 | 0.5024 | 59.72 | 0.56 | 0.9 | (0.0)† |
| | 0.5115 | 58.65 | 0.52 | 0.9 | |
| | 0.4894 | 61.30 | 0.43 | 0.7 | 0.7 |
| Goe-20 | 0.4860 | 61.73 | 0.41 | 0.7 | (0.0) |
| | 0.4983 | 60.21 | 0.40 | 0.7 | |
| | 0.4737 | 63.33 | 0.45 | 0.7 | 0.7 |
| Goe-51 | 0.4747 | 63.20 | 0.45 | 0.7 | (0.0) |
| | 0.4980 | 60.24 | 0.41 | 0.7 | |
| | 0.4912 | 61.08 | 0.03 | 0.0 | 0.0 |
| Goe-94 | 0.4960 | 60.49 | 0.03 | 0.0 | (0.0) |
| | 0.4812 | 62.35 | 0.03 | 0.0 | |
| ***Triglycine*** | | | | | |
| | 0.4887 | 73.66 | 0.47 | 0.6 | 0.7 |
| Goe-00 | 0.5025 | 71.64 | 0.51 | 0.7 | (0.1) |
| | 0.5089 | 70.74 | 0.44 | 0.6 | |
| | 0.4950 | 72.72 | 0.42 | 0.6 | 0.6 |
| Goe-20 | 0.4842 | 74.34 | 0.38 | 0.5 | (0.0) |
| | 0.4909 | 73.33 | 0.41 | 0.6 | |
| | 0.4887 | 73.66 | 0.39 | 0.5 | 0.5 |
| Goe-51 | 0.4740 | 75.94 | 0.43 | 0.6 | (0.0) |
| | 0.4812 | 74.81 | 0.41 | 0.5 | |
| | 0.4962 | 72.55 | 0.03 | 0.0 | 0.0 |
| Goe-94 | 0.4836 | 74.44 | 0.01 | 0.0 | (0.0) |
| | 0.4911 | 73.30 | 0.01 | 0.0 | |

† Standard deviation in parenthesis.

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